ACCESS TO CONVENIENT SYNTHESIS OF OXAZINONE AND PYRIMIDINONE HETEROCYCLES DERIVED FROM 2(3*H*)-FURANONE

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The conversion of the 2(3*H*)-furanone into the oxazinone and pyrimidinone derivatives are studied. A 2-(furan-2-ylmethylene)-4-oxo-4-phenylbutanoyl azide **3** was synthesized to carry out these conversions through its thermolysis in dry benzene and base-catalyzed decomposition of this azide in the presence of different amines. The structures of all compounds were demonstrated from their spectral data and elemental analyses.

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Introduction

In the last decades, our research group was interested in the conversion of 2(3*H*)-furanones into a variety of heterocyclic systems of synthetic and biological importance. Based on their facile ring opening by nitrogen nucleophiles, these furanones were converted into acyclic products which *via* ring closure afforded many heterocycles *viz*. pyrrolones, pyridazinones, triazoles, oxadiazoles, thiazolidinones, and benzoxazinones[1-17]. To our knowledge, the conversion of 2(3*H*)-furanones into pyrimidine derivatives has not been reported. Pyrimidine derivatives have a broad spectrum of biological activities. These derivatives are reported to have antiviral[18], antibacterial[19], anti-inflammatory[20], antimalarial[21], anticancer[22] and antihypertensive activities[23]. In view of the previous facts, we report herein the conversion of 2(3*H*)-furanones into oxazinone and pyrimidinone derivatives.

Experimental section

General

Melting points were measured on Gallen Kamp electric melting point apparatus. The IR spectra were recorded using potassium bromide disks on Fourier transform infrared Thermo Electron Nicolet 7600 spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) at the Central Laboratory of Faculty of Science, Ain Shams University. The ^{1}H-NMR spectra were run at 400 *MHz* on a GEMINI 400 BB NMR spectrometer (GEMINI, Manufacturing & Engineering Inc., Anaheim, CA, USA) using tetramethyl silane (TMS) as an internal standard in deuterated dimethylsulphoxide (DMSO-d_6) at the Main Defence Chemical Laboratory, Cairo. The mass spectra were recorded on Shimadzu GC-MS-QP-1000EX mass spectrometer (Shimadzu Scientific Instruments, Inc., USA) operating at 70 eV at the Microanalytical Center of Cairo University. The progress of all reactions was monitored by the thin layer chromatography using Merck Kiesel gel 60 F254 aluminum-backed plates.

General procedure for preparation of 4-(furan-2-ylmethylene)-6-phenyl-3,4-dihydro-2*H*-1,3-oxazin-2-one (4)

A solution of the azide **3** (5 mmol) in dry benzene (20 mL) was heated under reflux for one hour. The separated solid after cooling was collected by filtration and recrystallized from petroleum ether, 80-100/benzene, (2:1) to afford oxazinone **4** as red crystals; mp. 170-172ºC, yield 49%. IR (KBr) (ν, cm^{-1}): 3175 (NH), 3057, 3040 (Aryl-H), 1732 (C=O$_{Oxazinone}$), 730, 684 (δ_{5H}). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 13.28 (s, 1H, OH, *Lactim form*, D_2O-*Exchangeable*), 13.25 (s, 1H, NH, *Lactam form*, D_2O-*Exchangeable*), 8.10-6.91 (m, 8H, Ar-H), 6.78 (s, 1H, C-H$_{Oxazinone}$), 6.48 (s, 1H, CH=). MS (*m/z*, %): 253 ($M^{+\cdot}$, 46), 252 (100), 222 (62), 194 (67), 115 (34), 104 (23), 81 (36),77 (64). Anal. Calcd. for $C_{15}H_{11}NO_3$ (253.07): C, 71,14; H, 4.38; N, 5.53. Found: C, 71.08; H, 4.27; N, 5.42.

Pyrolysis of azide 3

In 10 mL round-bottomed flask fitted with an air condenser was placed 0.5 g of the azide **3**. The flask was heated on a sand bath whereby the azide was fused. Heating was continued for another one hour. The product obtained after recrystallization from petroleum ether, 80-100/ benzene, (2:1) was proved by direct comparison (mp., mixed mp. and TLC) to be the oxazinone **4**.

Reaction of the azide 3 with benzylamine

Benzylamine (2 mmol) was added dropwise to a solution of azide **3** (2 mmol) in dry benzene (20 mL) and the mixture was stirred at room temperature for 2 h or heated under

reflux for one hour. The precipitated solid was collected by filtration and recrystallized from petroleum ether, 80-100/benzene (3:1) to give benzylurea **5** and *N*-benzyl-pyrimidine **6**, respectively.

1-Benzyl-3-(1-(furan-2-yl)-4-oxo-4-phenylbut-1-en-2-yl)urea (5)

Brown crystals, mp. 195-197ºC, yield 52%. IR (KBr) (ν, cm^{-1}): 3247 (NH), 3059, 3028 (Aryl-H), 2937, 2922 (Alkyl-H), 1671 ($C=O_{Ketone}$), 1643 ($C=O_{Amide}$), 759, 692 (δ_{5H}). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 9.51 (s, 1H, NHCO, *D_2O-Exchangeable*), 9.20 (s, 1H, NHCH$_2$, *D_2O-Exchangeable*), 7.75-7.05 (m, 13H, Ar-H), 6.73, 6.56 (*Two singlets*, 1H, *E*- & *Z*-isomers, CH=), 4.33 (s, 2H, NHCH$_2$), 3.19-3.01 (dd, 2H, CH_2CO, *J*= 16.4 *Hz*). MS (*m/z*, %): MS (*m/z*, %): 360 ($M^{+\cdot}$, 10), 253 (42), 210 (50), 192 (60), 106 (3), 91 (100), 77 (51). Anal. Calcd. for $C_{22}H_{20}N_2O_3$ (360.15): C, 73.32; H, 5.59; N, 7.77. Found: C, 73.21; H, 5.43; N, 7.68.

1-Benzyl-4-(furan-2-ylmethylene)-6-phenyl-3,4-dihydro-pyrimidin-2(1*H*)-one (6)

Yellow crystals, mp. 148-150ºC, yield 47%. IR (KBr) (ν, cm^{-1}): 3259 (NH), 3065, 3021 (Aryl-H), 2955, 2935 (Alkyl-H), 1652 (C=O), 738, 692 (δ_{5H}). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 10.04 (s, 1H, OH, *Lactim form*, *D_2O-Exchangeable*), 10.03 (s, 1H, NH, *Lactam form*, *D_2O-Exchangeable*), 7.75-7.05 (m, 13H, Ar-H), 6.83 (s, 1H, C-H$_{Pyrimidine}$), 6.66 (s, 1H, CH=), 4.81 (s, 2H, CH_2). MS (*m/z*, %): 342 ($M^{+\cdot}$, 100), 251 (40), 223 (55), 208 (42), 166 (29), 91 (80), 77 (32). Anal. Calcd. for $C_{22}H_{18}N_2O_2$ (342.14): C, 77.17; H, 5.30; N, 8.18. Found: C, 77.08; H, 5.21; N, 8.02.

Reaction of the azide 3 with hydrazine: Synthesis of *N*-(1-(furan-2-yl)-4-oxo-4-phenylbut-1-en-2-yl)hydrazine-carboxamide (7)

Hydrazine (2 mmol) was added dropwise to a solution of azide **3** (2 mmol) in dry benzene (20 mL) and the mixture was stirred at room temperature. The obtained solid was collected by filtration and recrystallized from benzene to give the semicarbazide derivative **7** as brown crystals, mp. 205-207ºC, yield 39%. IR (KBr) (ν, cm^{-1}): 3331, 3208 (NH, NH_2), 3064, 3032 (Aryl-H), 2923, 2859 (Alkyl-H), 1700 ($C=O_{Ketone}$), 1659 ($C=O_{Amide}$), 743, 699 (δ_{5H}). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 9.01 (s, 1H, NH, *D_2O-Exchangeable*), 7.34 (s, 1H, NHNH$_2$, *D_2O-Exchangeable*), 7.75-6.72 (m, 8H, Ar-H), 6.56, 6.45 (*Two singlets*, 1H, *E*- & *Z*-isomers, CH=), 4.50 (s, 2H, NH_2), 3.19-3.00 (dd, 2H, CH_2, *J*= 16.4 *Hz*). MS (*m/z*, %): 285 ($M^{+\cdot}$, 14), 255 (39), 227 (21), 150 (12), 180 (10), 119 (20), 77 (100). Anal. Calcd. for $C_{15}H_{15}N_3O_3$ (285.11): C, 63.15; H, 5.30; N, 14.73. Found: C, 63.04; H, 5.19; N, 14.61.

Synthesis of 1-amino-4-(furan-2-ylmethylene)-6-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (8)

A solution of the azide **3** (2 mmol) and hydrazine (2 mmol) in dry benzene (20 mL) was heated at 90ºC for one hour. The reaction mixture was concentrated and then cooled. The obtained solid was collected by filtration and recrystallized from petroleum ether, 60-80/benzene (2:1) to give 1-aminopyrimidinone derivative **8** as faint brown crystals, mp. 160-162ºC, yield 35%. IR (KBr) (ν, cm^{-1}): 3330, 3204 (NH, NH_2), 3063, 3032 (Aryl-H), 2959, 2939 (Alkyl-H), 1659 (C=O), 743, 699 (δ_{5H}). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 13.10 (s, 1H, NH, *D_2O-Exchangeable*), 7.94-6.81 (m, 8H, Ar-H), 6.69 (s, 1H, CH$_{Pyrimidine}$), 6.39 (s, 1H, CH=), 4.99 (s, 2H, NH_2). MS (*m/z*, %): 267 ($M^{+\cdot}$, 100), 239 (21), 162 (28), 102 (19), 81 (15), 77 (30). Anal. Calcd. for $C_{15}H_{13}N3O_2$ (267.10): C, 67.40; H, 4.90; N, 15.72. Found: C, 67.28; H, 4.75; N, 15.58.

Reaction of the azide 3 with *p*-toluidine; Synthesis of 1-(1-(furan-2-yl)-4-oxo-4-phenylbut-1-en-2-yl)-3-*p*-tolylurea (9)

p-Toluidine (2 mmol) was added to a solution of the azide **3** (2 mmol) in dry benzene (20 mL) and the mixture was heated on water bath for 2 h. The obtained solid was collected by filtration and recrystallized from petroleum ether, 80-100/benzene (2:1) to give *p*-tolylurea derivative **9** as brown crystals, mp. 220-222ºC, yield 49%. IR (KBr) (ν, cm^{-1}): 3316, 3209 (2 NH), 3088, 3025 (Aryl-H), 2915, 2858 (Alkyl-H), 1699 ($C=O_{Ketone}$), 1650 ($C=O_{Amide}$), 733, 687 (δ_{5H}). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 9.80 (s, 1H, NH$_{Tolyl}$, *D_2O-Exchangeable*), 9.05 (s, 1H, NH, *D_2O-Exchangeable*), 7.75-6.83 (m, 8H, Ar-H), 7.19-7.17 (d, 2H, H$_{Tolyl}$, *J*= 8 *Hz*), 7.05-7.03 (d, 2H, H$_{Tolyl}$, *J*= 8 *Hz*), 6.73, 6.56 (*Two singlets*, 1H, *E*- & *Z*-isomers, CH=), 3.20-3.05 (dd, 2H, CH_2, *J*= 16 *Hz*), 2.27 (s, 3H, CH_3). MS (*m/z*, %): 360 ($M^{+\cdot}$, 51), 345 (41), 270 (25), 193 (15), 119 (28), 91 (100), 77 (50). Anal. Calcd. for $C_{22}H_{20}N_2O_3$ (360.15): C, 73.32; H, 5.59; N, 7.77. Found: C, 73.20; H, 5.42; N, 7.64.

Synthesis of 4-(furan-2-ylmethylene)-6-phenyl-1-*p*-tolyl-3,4-dihydropyrimidin-2(1*H*)-one (10)

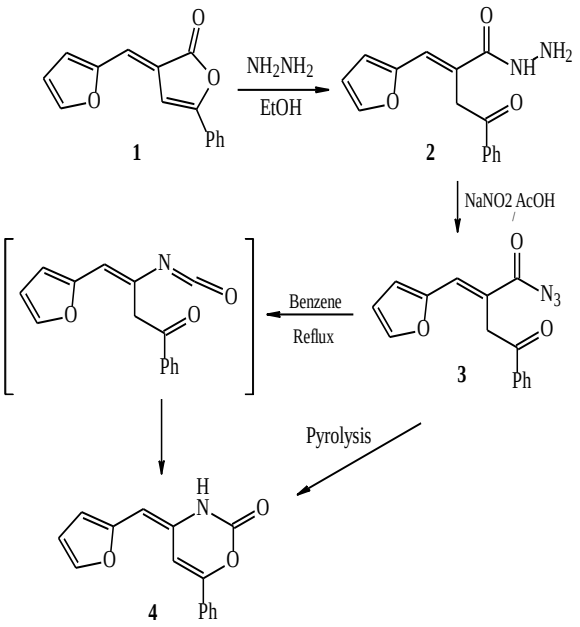
A solution of the azide **3** (2 mmol) and *p*-toluidine (2 mmol) in dry benzene (20 mL) was heated at 90ºC for 6 h. The obtained solid after cooling was collected by filtration and recrystallized from benzene to give *N*-*p*-tolylpyrimidine derivative **10** as orange crystals, mp. 178-180ºC, yield 38%. IR (KBr) (ν, cm^{-1}): 3317 (NH), 3061, 3025 (Aryl-H), 2974, 2918 (Alkyl-H), 1649 (C=O), 733, 687 (δ_{5H}). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 12.16 (s, 1H, NH, *D_2O-Exchangeable*), 7.94-7.02 (m, 8H, Ar-H), 7.27-7.25 (d, 2H, H$_{Tolyl}$, *J*= 8 *Hz*), 7.08-7.06 (d, 2H, H$_{Tolyl}$, *J*= 8 *Hz*), 6.68 (s, 1H, C-H$_{Pyrimidine}$), 6.38 (s, 1H, CH=), 2.33 (s, 3H, CH_3). MS (*m/z*, %): 342 ($M^{+\cdot}$, 40), 327 (100), 252 (50), 146 (18), 102 (10), 90 (21), 77 (100). Anal. Calcd. for $C_{22}H_{18}N_2O_2$ (342.14): C, 77.17; H, 5.30; N, 8.18. Found: C, 77.05; H, 5.18; N, 8.04.

Results and discussion

Conventionally, 2-(furan-2-ylmethylene)-4-oxo-4-phenyl-butanoyl azide **3** was previously prepared *via* the following two steps:

(i) The 2(3*H*)-furanone **1** reacted with hydrazine hydrate at room temperature in ethanol to give the acid hydrazide **2**.

(ii) The hydrazide was converted into the corresponding acyl azide **3** by the action of sodium nitrite in AcOH[7] (Scheme 1).



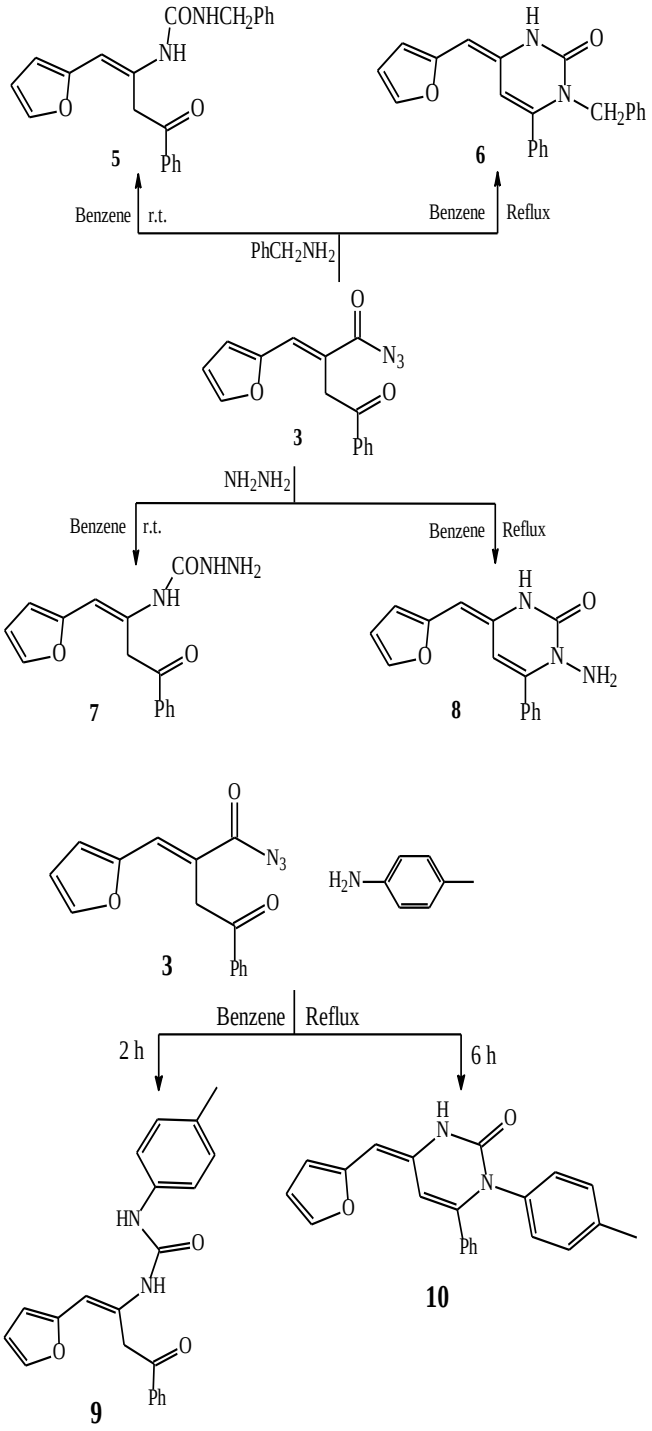
Scheme 1. Synthesis of compound **4**.

The structure of **3** was inferred from its IR spectrum which displayed ν N_3 of azide at 2142 cm^{-1} and devoid from NH & NH_2 absorption bands.

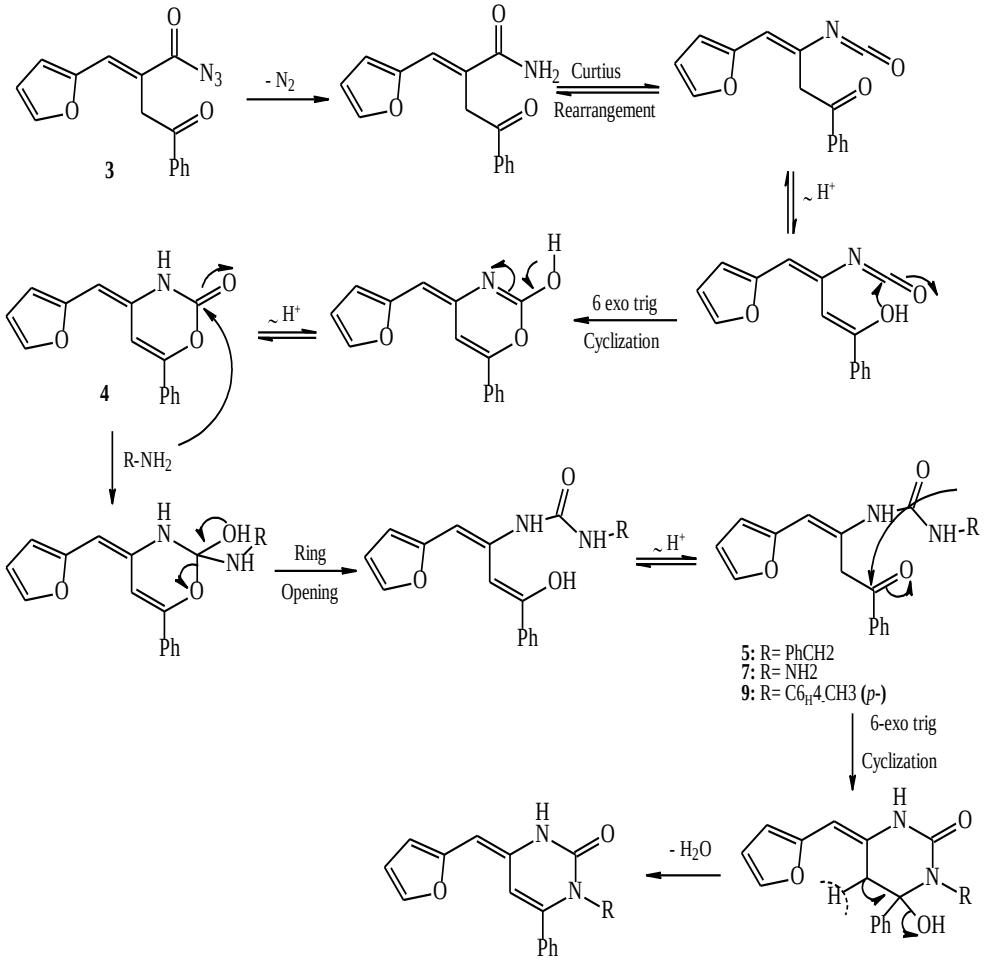
Acyl azides are known to be suitable candidates for the synthesis of many acyclic and heterocyclic derivatives *via* their acid or base-catalyzed decompositions. Thus, it was of interest to the authors to construct oxazinone and pyrimidinone derivatives using the azide **3** as a key starting material. The reactions of **3** with benzylamine, hydrazine, and *p*-toluidine were studied (Scheme 2).

Thermolysis of the azide in dry benzene led to the construction of oxazinone derivative **4** as sole product (Scheme 1). The formation of **4** can be postulated *via* loss of nitrogen gas then Curtius rearrangement of the resulting nitrene to afford isocyanate intermediate (non-isolable) followed by exo-trig ring closure (**Scheme 3**). The structure of **4** was substantiated from its IR spectrum which lacked ν N=C=O of isocyanate group and only displayed the stretching absorption bands of oxazinone CO and NH at ν 1732 & 3175 cm^{-1}, respectively. The ^{1}H-NMR spectrum was a real evidence for its existence as a mixture of lactam-lactim tautomers through which two signals at δ 13.28 and 13.25 ppm displayed, both integrated to one exchangeable proton of NH-CO ⇌ N=C-OH grouping. Moreover, the mass spectrum exhibited the correct molecular ion peak at *m/z* 253 (46%) as well as the base peak at *m/z* 252 (100%) representing [M-1] cation. The same product **4** was obtained *via* pyrolysis of the azide by fusion in neat.

When azide **3** was allowed to react with benzylamine in dry benzene at room temperature, the benzylurea derivative **5** was obtained, while the reaction in refluxing benzene for 1 h resulted in the formation of pyrimidinone derivative **6**. The structures of **5** & **6** were deduced from their analytical and spectral data. The IR spectrum of **5** revealed a broad band at 3247 cm^{-1} attributable to NH groups, as well as, the characteristic absorption bands for C=O of ketone and amide groups at 1671 & 1643 cm^{-1}, respectively. The ^{1}H-NMR spectrum of **5** showed that CH_2CO protons were magnetically non-equivalent which may be attributed to hydrogen bonding between PhC=O and NH (Cf. Experimental). The ^{1}H-NMR spectrum of **6** was devoid from the splitting pattern of CH_2CO group and showed a singlet band at δ 4.8 ppm due to benzyl-CH_2 protons, as well as, two signals at δ 10.03 and 10.04 ppm, both integrated to one exchangeable proton of NH-CO ⇌ N=C-OH grouping.



Scheme 2. Reactions of azide **3**



Scheme 3. Formation of the oxazinone and pyrimidinone derivatives.

The reaction of **3** with hydrazine was also dependent on the reaction conditions. Thus, stirring **3** with hydrazine in benzene at room temperature afforded the semicarbazide derivative **7** while at refluxing conditions; 1-amino-pyrimidinone derivative **8** was produced. The IR spectrum of **7** showed a broad band at 3331 & 3208 cm^{-1} attributable to NH & NH_2 groups, as well as, the characteristic absorption bands for C=O of ketone and hydrazide groups at 1700 & 1659 cm^{-1}. The ^{1}H-NMR spectrum of **7** exhibited geminal coupling of two magnetically non-equivalent protons of CH_2 (*J*=16.4 *Hz*) as evidence for the aforementioned hydrogen bonding (Cf. Experimental).

The aromatic amine, *p*-toluidine, failed to affect decomposition of the azide **3** at room temperature, a behavior that may be attributed to the weak nucleophilicity of the *p*-toluidine nitrogen atom. But, when the reaction was carried out in dry benzene at 90ºC for 2 h, Base-catalyzed decomposition occurred with the formation of the *p*-tolylurea derivatives **9**. On the other hand, Carrying out the reaction under reflux conditions for 6 h led to the construction of *p*-tolylpyrimidinone derivatives **10**. The IR spectrum of **9** provided the characteristic absorption bands for two NH & two C=O of ketone and amide groups at 3317 & 3209 cm^{-1} and 1699 & 1650 cm^{-1}. Also, the geminal coupling between CH_2 protons (*J*=16.0 *Hz*) was clearly shown in its ^{1}H-NMR spectrum indicating to hydrogen bonding (Cf. Experimental). The formation of the oxazinone and pyrimidinone derivatives may be represented by

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ARUM: A PLANT GENUS WITH GREAT MEDICINAL POTENTIAL

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Keywords: Arum, alkaloids, toxicity, anticancer, antibacterial, volatile amines

Plants belonging to the genus *Arum* are being used for nutritional and medicinal purposes for many centuries, despite their toxicity. Few subspecies of this genus were widely investigated by modern research, mainly for potential therapeutic goals and drug discovery. Other subspecies were never studied by current research despite the fact that some of them have known and well documented traditional medicinal and other uses. In this review article, we will present the traditional uses of this plant genus and summarize the published results of modern medicinal and other studies of these plants. Special attention will be drawn to effective, natural products that were isolated from these plants. The toxicity of the plants will be discussed extensively.

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Introduction

The genus *Arum* (Araceae) is native to Asia, Europe, and northern Africa. The number subspecies of this genus is not definite: while some researchers consider 29 subspecies,[1] the "U.S. National Plant Germplasm System" counts 44.[2] However, the number of subspecies that have known (reported) traditional uses and were reported in current studies for biological/medicinal activities hardly exceeds two dozens. Archeological evidence indicate uses of *Arum* by humans since ancient times.[3]

Arum subspecies are well known for their *thermogenesis*.[4] This is to say that alteration of light and dark in the environment of the plant stimulates the primordia of the male plant to produce salicylic acid that triggers thermogenic reactions. For some subspecies like *A. italicum* and *A. maculatum*, the temperature of the flower can be higher by 15-25 °C than the surrounding air. This phenomenon is one of two major pollination strategies that aim to attract potential pollinators like insects. The other strategy is releasing a very strong odor that attracts insects. In most subspecies of *Arum*, this odor is foul (dung, *A. palaestinum*, *A. dioscoridis*, *A. elongat*ed and others) but in some subspecies, it can be from not perceptible (*A. jacquemontii*) to even pleasant (*A. gratum*).[4] In addition to many volatile amines that will be presented in next sections, many compound families are represented in these pollination odors.[4,5] Some important compounds are shown in Figure 1.

Detailed study of the floral odor of *A. italicum* was published earlier in 2004, where several methods of isolation and trapping volatile compounds were used.[6] In this study, very similar results were obtained comparing with the previously cited publications (4,5), and only stereochemical and structural isomerization can be noticed, comparing with the compounds shown in Figure 1.

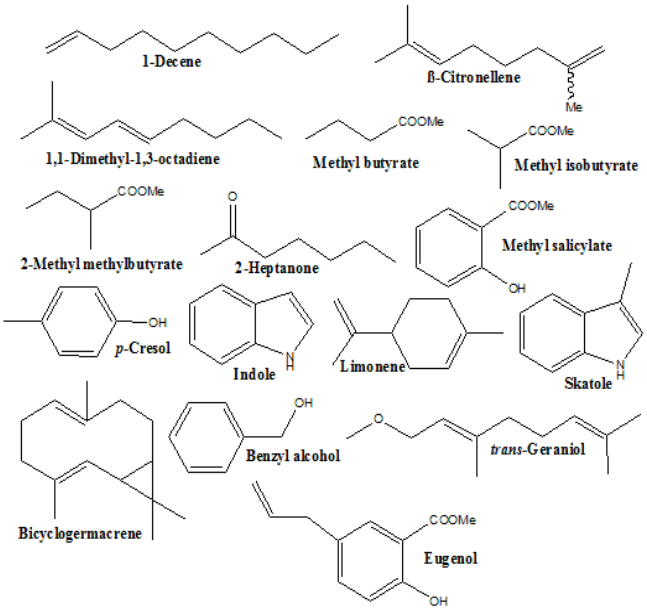

Figure 1. Major compounds that compose the pollination odors of *Arum* subspecies

Traditional uses of Arum subspecies

Most listed subspecies of *Arum* have no documented traditional uses. Most of this group was not reported by modern research studies as well. But scanning the literature of *Arum* traditional uses reveals two significant findings:

a) The vast majority of documented traditional users are aware of the *toxicity* of these plants and in

Most texts this property is mentioned and potential users are explicitly warned. Modern research approved this as shown in below (discussion).

b) It is notable that the most important traditional uses of *Arum* are for nutritional purposes then by medicinal use as an anticancer agent. This using field is also in agreement with current research results.

Table 1. Traditional uses of *Arum* subspecies

***Arum* subspecies**	**Country/Region**	**Used parts**	**Uses;Admistration (References)**
A. balansanum	Bulgaria	Tubers	Against haemorrhoids, direct contact[7]
A. conophalloides	Iran	Leaves	Food, flavor, elimination of seasonal allergies[8]
	Turkey	Leaves	Food (*Srama*)[9]
A. cyrenaicum	Libya	Corms	Food, ornaments[10]
A. detruncatum	Bulgaria	Tubers	Against hemorrhoids, direct contact[7]
	Turkey	Leaf/Root	Anti-diarrhea, kidney stones, stomachic; infution (internal)/ Antidiabeteic; decoction (internal)[11]
A. dioscorides	India	Stems	Boils rure; aqueous extract, direct contact[12]
	Jordan	Leaves	Anticancer; decoction[13]
	Palestine	NS*	Cancer, prostate disorders[14]
	Palestine	Leaves	Anticancer: liver, stomach; decoction (detailed)[15]
	Turkey	Leaves	Food (*Srama*)[9]
	Turkey	Roots, flowers	Treatment of inflamed woinds; poultice, to cure hemorrhoids; direct contact[16]
A. elongatum	Bulgaria	Tubers	Against hemorrhoids, direct contact[7]
	Turkey	Leaves	Abdominalpain, antihypertensive, antidiabetic, rheumatism; infusion, compress or drink[17]
	Turkey	Tuber	Haemorrhoids; tubers is crushed to powder and consumed[18]
A. italicum	Iraq	Leaves	Food[19]
	Italy	Leaves	Anti-warts; topical applical[20,25]
	Italy	Tubers, leaves	Food for pigs[21,22]
	Italy	Rhizomes	To heal contusions; pieces are locally applied[23]
	Italy	NS	Vesicatory, treatment of CNS disorders; NS[24]
	Italy	Leaves, rhizomes	Rheumatic pains; leaves and rizhomes macerated in oil[26]
	Italy	Tubers, leaves	Against warts, rheumatic pains[7]
	Slavic culture (Italy)	Leaves, tubers	Food, unclear; NS[27]
	Spain	Leaves, tubers	Skin, muscles, skeleton; very detailed[28]
	Spain	Spathe	Ludic; NS[29]
	Tunisia	NS	Vesicatory, treatment of CNS disorders; NS[24]
	Turkey	Flowers/Tubers/ Tubers & fruits	Hemorrhoids; decoction/Wonen diseases, cancer, eczema; decoction, decoction, consumed/ hemorrhoids; consumed[30]
	Turkey	Tubers	Treatment of hemorrhoid, expectorant; infusion[31]
	Turkey	Leaves	Food (soup); boiled[32]
	Turkey	Tubers/aerial parts	Treat hemorrhoid; crushed, direct contact/ hepatitis, muscle pain; crushed, decoction[33]
	Turkey	Tubers	Food; boiled/ Treat hemorrhoid, eczema; boiled[34]
A. hygrophilum	Jordan	Leaves	Anticancer; decoction[13]
A. maculatum	Bulgaria	Tubers	Against haemorrhoids, direct contact[7]
	Czech Republic	Rhizomes	Food; boiled[35]
	Europe	NS	Antimalarial; NS[36]
	Iraq	Leaves	Treat intestinal worms, rheumatism; decoction[37]
	Italy	NS	NS;NS[38]
	Turkey	NS	Anti colitic, abortive; NS[39]
	Turkey	Corms	Treat hemorrhoids; crushed and swallowed[40]
	Turkey	Leaves	Food; eaten fresh in salads[41]
	Turkey	Leaves	Food (*Srama*); cooking stuffed leaves[9]
A. palaestinum	Greco-Arab region	Leaves	Anticancer, urinary disorders; NS[42]
	Greco-Arab region	Leaves	Anticancer (especially colon), internal bacterial infections, poisoning, disturbances of the circulatory system.; cooking[43]
	Islamic-Arab region	NS	Anticancer; NS, but modern results presented[44]
	Jordan	Leaves	Anticancer; decoction[13]
	Jordan	Leaves	Anticancer; cooked with oinion ans salt[45]
	Lebanon	Leaves	Rheumatism; decoction, maceration[46]

.Table 1 cont

	Middle-East	NS	Anticancer; NS, links modern research[47]
	Middle-East	NS	Anticancer; NS, links to modern homeopathy[48]
	Palestine	Leaves, flowers	Internal bacterial infection, cancer, poisoning, circulatory system; decoction (detailed)[49]
	Palestine	NS	Anticancer; NS[50]
	Palestine	Leaves	Food; detailed procedure[51]
	Palestine	Leaves	Anticancer: liver, colon, kidney, breast; decoction (detailed)[15]
NS	Turkey	Roots/flowers	Rheumatism; pounded/ treat oxyuris; NS[52]

*NS, Not specified

In Table 1, we summarize the traditional uses of *Arum*, arranged by regions/countries. Roots and fruits of the plants are very toxic, so it's highly recommended to pay attention to the plant parts used.

Modern research reports of *Arum* subspecies

Many *Arum* subspecies were studied so far, where the most investigated are *A. dioscorides*, *A. maculatum*, and *A. palaestinum*. It is interesting to see that unlike other plant families that are used by humans for millenia, modern research of *Arum* started just little more than three decades ago, while other plant families are being studied for much longer periods of time. Many medicinal and other biological activities of *Arum* plants were reported. In Table 2 a summary of these reports is presented.

Discussion

Arum subspecies are known and used by humans since ancient times. But new subspecies are still identified once in a while. *Arum megobrebi* was identified and classified as wild subspecies of *Arum* that grows in Turkey and Georgia.[107]

Reading data in Tables 1 and 2 reveals a wide variety of activities of the genus *Arum*. But it is crucial to notice that many *Arum* subspecies were never mentioned for traditional uses or reported by recent research publications. These include *A. alpinariae*, *A. besserianum*, *A. byzantinum*, *A. concinnatum*, *A. cylindraceum*, *A. gratum*, *A. hainesii*, *A. jacquemontii*, *A. lucanum*, *A. megobrebi*, *A. pictum*, *A. purpureospathum*, *A. rupicola*, *A. sintenisii*. It is evident as well that modern research has studied (so far) more *Arum* subspecies than those that were documented as having traditional uses. Most investigated subspecies is *A. palaestinum*.

It is interesting to pay attention to *A. cyrenaicum*, an endemic subspecies that grows wild only in Libya. In reference 8, authors report two traditional uses of this plant (food and ornaments), and it is interesting to notice that the used parts are corms, not leaves, contrary to most *Arum* subspecies, where corms are highly toxic. But these authors have mistakenly classified this plant into the Poaceae family, while the correct classification is in the Araceae family.[55]

One of the most important properties of *Arum* that was consistently mentioned by traditional users and approved by modern research is the toxicity of these plants. Despite being recommended for use as food and medicine, the toxicity of *Arum* is indicated in most texts.[108] Modern reports rank *Arum* subspecies as one of the most important causes of children poisoning in Brazil.[109] Among these, *A. italicum* is responsible for the largest number of poisoning cases, and all parts of the plant are toxic.

Toxicity of *Arum* subspecies results from several single compounds or compound families. Calcium oxalate is one of the primary toxic compounds in *Arum* plants,[55] but it decomposes with cooking. The same occurs to cyano glycosides such as triglochinin,[76] a toxic compound present in *Arum*, that its structure is shown in **Figure 2**

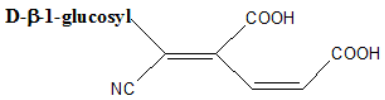

Figure 2. Structure of triglochinin

Among reported *Arum* subspecies in the toxic context, *A. maculatum* is the most published by current research publications so far. One of the earliest reports was published in 1861, and it presents some poisoning cases.[110] Part of this toxicity is due to the presence of toxic odorants, especially volatile amines.[111] In addition to oxalates and cyano compounds, the toxicity of *A. maculatum* is intensified by alkaloids and saponins.[112] The orange-colored fruits of *A. maculatum* are very attractive yet very poisonous, and they are responsible for most poisoning events caused by this plant.[113] The toxicity of *A. palaestinum* is also known and published: ethanolic extract of the plant was found toxic to the liver of female rats.[114] Despite that, unlike other natural, plant-derived anticancer therapies, *A. palaestinum* has no herb-drug contradictions with synthetic drugs.[115]

An interesting modern research report presented in **Table 2** is about *A. Conophalloides*.[54] All 18 compounds identified in the essential oil of this plant do not contain nitrogen. No amines or alkaloids. This situation can be understood from two reasons: nitrogen containing compounds, especially amines are volatile and alkaloids are not volatile and mostly water soluble, so they are not present in the essential oil that contains mainly hydrophobic compounds.

Table 2. Overview of modern research finding of *Arum* studies

***Arum* subspecies**	**Activity/property**	**Major findings (References)**
A. apulum	Odor components	Traces of indole, terpinolene[53]
A. creticum	Odor components	Presence of benzaldehyde, benzyl alcohol, indole[53]
A. Conophalloides	Essential oil	18 Compounds were found including (>5%): nonanal, β-ionone, *T*-cadinol, *T*- muurolol, fitone, methyl palmitate[54]
A. cyrenaicum	Odor components	Traces of indole[53]
	Antioxidant, toxicity	Moderate antioxidant activity (DPPH), toxicity is a result of the presence of calcium oxalate and cyanogenic glycosides[55]
A. dioscorides	Flowering amines	Methylamine, skatole[56]
	Antilipoperoxidation	Both aqueous and methanolic extracts showed modertare antioxidant capacity[57]
	Fatty acids in seeds	Methanolic and acetone extracts were prepared and their antioxidant capacities were tested by three methods. Methanolic extract showed higher capacity and its full fatty acid composition is presented[58]
	Antioxidant, composition	Three extracts were prepared and their antioxidant capacities (DPPH) were tested. Methanolic was highest and contained highest number (5) of tested antioxidants[59]
	Antimicrobial	Four extracts were prepared: water, ethanol, methanol and acetone. Testing against 6 moicrobes showed that aqueous extract was most active[60]
	Antibacterial	Ethanolic extract was tested against 6 bacteria types: weak[61]
	Minerals content	Magnesium, 24.6 g/Kg, sulfur 39.4 (cites other studies)[62]
	Antioxidant	Four extracts (water, ethanol, methanol, acetone) were prepared and tested for reducing power (ferric, ethanolic highest) and scavanging of DPPH (methanolic highest)[63]
	Enzyme inhibition	Ethanolic and aqueous extracts were tested for *in vivo* and *in vitro* enzyme inhibistion. Both were active as inhibitors of gastrointestinal enzymes involved in carbohydrate and lipid digestion and absorption[64]
	Antioxidant activity	Methanolic and aqueous extracts were prepared, analyzed for phenolic content and tested for antioxidant activity. Methanolic extract was more active[65]
A, elongatum	Chemical composition	Minerals were quantified. Iron highest (134 mg/Kg)[66]
	Antioxidant activity	Methanolic and aqueous extracts were prepared, analyzed for phenolic content and tested for antioxidant activity. Methanolic extract was more active[65]
A. euxinum	Macroelement content	Macroelement (N, P, K) content was quantified during different growth phases. Various localities were also tested[67]
	Antibacterial	Aqueous, ethanolic and methanolic extracts were tested for antibacterial activity: ethanolic>methanolic. Aqueous inactive[68]
A. hygrophilum	Antimicrobial	95% Ethanol-water extract was tested for antimicrobial activity: active against some species and inactive against others[69]
	Antimicrobial	Four extracts were prepared: water, ethanol, methanol and acetone. Testing against 6 moicrobes showed only methanolic and ethanolic extracts were active just against *C. albicans*[60]
	Antioxidant activity	Methanolic and aqueous extracts were prepared, analyzed for phenolic content and tested for antioxidant activity. Methanolic extract was more active[65]
A. idaeum	Odor components	Presence of benzaldehyde, benzyl alcohol, nonanal[53]

.Table 2 cont

A. italicum	Flowering amines	Isobutylamine, dietlhylamine, ethylamine, dimethylamine, methylamine, 2-aminoethanol, 1,2-propanediamine, 1,6-hexanediamine, agmatine, cadaverine, histamine, putrescine[56]
	Hydroperoxysterols	In addition to known sterols and hydroperoxysterols, 6 new of the second class were isolated and characterized[70]
	Carotenoids	Ethanolic extract of the fruits was prepared during maturation and ripening stages. 18 different carotenoids were isolated and identified, along with chlorophyl precursor (*cis*-OH-phytoene), chlorophylls A and B, and chlorophyll-like, pheophythin[71]
	Accumulation of metals	Among 13 plant species, *A. italicum* was best accumulator of Zn^{+2}, Cd^{+2} and Cu^{+2}. It was not successful with Pb^{+2}. This suggests a bioremediation method of contaminated soils[72]
	Fatty acids in seeds	Essential oil of the plant was isolated and fatty acids were methylated (esters, BF_3/MeOH), isolated and analyzed. 21 acids were found from caprylic (C8:0) to legnoceric (C24:0)[73]
A. korolkowii	Lipids in tubers	Hydrophobic compounds were isolated by column chromatography. Composition is reported by groups[74]
A. maculatum	Odor components	Presence of indole, nonanal, α-pinene, β-pinene, tepinolene, α-Copaene[53]
	Unsaponifiable lipids	Spadices were treated with concentrated base and the hydrosylate was extracted with ether, resulting long chain hydrocarbons, long chain alcohols and carotenoids[75]
	Triglochinin in spathes	The toxic cyanogycoside was isolated and identified[76]
	Fatty acids contents	Fatty acids of seed oil were isolated by picolinyl esterification and purification. In addition to medium chain acids (C14:0), acids with aromatic residues (including pyridyl) were detected[77]
	Pro-inflammatory	A monocot lectin (ptotein) was isolated from the tubers of the plant. It acts as agglutinin and has pro-inflammatory activity[78]
	Insecticidal	Mannose binding lectin was isolated from the tubers. It binds to the glycosylated insect gut receptors[79]
	Cytogenetic	Aqueous extract inhibited cell mitosis of bone marrow of mice[80]
	Antioxidant capacity of food	Antioxidant capacity of leaves were tested in three forms: fresh, powder and stored. All forms showed similar capacities[81]
	Analgesic	Aqueous extract analgesic activity was compared with that of declofenac-Na and morphine. It was more active than the first and had similar activity of the second[82]
	Antibacterial	Ethanolic extract tested against 7 typyes of bacteria: weak[83]
	Antibacterial	Four extracts (petroleum ether, chloroform, ethyl acetate and 70% methanol) of aerial parts were tested against two bacteria. Hydromethanolic extract showed highets activity[84]
	Antioxidant	Methanolic extract of whole plant was by DPPH assay and found very active, even more than ascorbic acid[85]
	Essential oil, antibacterial, antioxidant	Essential oil was tested for antibacterial (3 bacteria) and antioxidant (DPPH) activities. Composition found: palmitic acid 23.31 %, phytol 13.02 %, methyl 9,12,15-octadecatrienoate 10.34 %, methyl linolenate 8.64 % [86]
A. nigrum	Odor components	Presence of indole[53]
A. orientale	Glucomannan	Major glucomannan (with other minor polysaccharides) was isolated from the tubers. It is composed of D-glucose and o-mannose (2: 3.1), and traces of uronic acid[87]
A. palaestinum	Isoorientin	Isoorientin (luteolin 6-C-glucoside) that was isolated by soaking aerial parts in ethanol, then partitioned and chromatographed. The compound myolytic activity on smooth muscle-containing preparations from the rat and the guinea-pig[88]
	Piperazirum	Piperazirum was isolated and characterized as a novel alakloid (1 in Figure 6 in the discussion section)[89]

..Table 2 cont

A. palaestinum	New Alkaloid	(S)-3,4,5-trihydroxy-1H-pyrrol-2(5*H*)-one (2 in Figure 6) was isolated from the aqueous extract and characterized. The ethyl acetate extract showed strond antioxidant and sufficient anticancer activities[90]
	Antioxidant, antidiabetic	Aqueous and methanolic extracts were prepared and tested for antioxidant activity (DPPH): moferate. This result is in agreement with total phenolics content and antidiabetic traditional use[91]
	Anticancer modern herbal medicine	Aerial parts, especially leaces are used as anticancer agents in modern herbal Palestinian medicine. It is used raw, cooked (food) or as a decoction[92]
	Anticancer, antioxidant	Aqueous and ethanolic extracts were tested for anticancer and antioxidant activities. Anticancer was very strong (aqueous >> ethanolic), antioxidant was weak (aqueous > ethanolic)[93]
	Diketopiperazines	Two new alkaloids were isolated from the aqueous extract and characterized. Only 3 in Figure 6 showed cytotoxic activity[94]
	Antimicrobial	70% Aqueous ethanol extract was tested for antibacterial activity against six types of bacteria (weak), and for antidermatophyte activity (2 fungi): moderate[95]
	Phenolics, protein	70% Aqueous methanol extract was analyzed for proteins and phenolics[96]
	Phthalates	Three phthalates isolated from ethanolic extract: Diisobutyl phthalate, di-n-propyl phthalate, di-n-octyl phthalate[97]
	Antioxidant, antitumor	Ethanolic extract was tested for antioxidant activity: moderate. The title of this publication include *in vitro* antitumor testing but this does not exist in the article[98]
	Phenolics	Phenolic extract was analyzed for phenolic compounds[99]
	Metabolites of leaves	Comprehensive metabolite profiling of Arum liquid chromatography–tandem mass spectrometry (UHPLC–DAD-ESI-MS/MS) revealed 191 compounds, with detailed analysis of selected entries[100]
	Partial composition and antioxidant	Concentrations of minerals, phenolic and anthocyanins were determined, and antioxidant (DPPH) capacity of methanolic extract of leaves was tested (high)[101]
	Cytotoxic	Ethereal and ethyl acetate extrracts as well as well as four flavonoids isolated from the plant, showed significant antiproliferative activity[102]
	Antioxidant, Anti-inflammatory, Anti-diarrheal	Ethanolic extract was prepared from the plant along with other plants. Total phenolic was determined (low), antioxidant capacity (DPPH, moderate), anti-inflammatory (moderate) and anti-diarrheal (inactive)[103]
	Locality influence on content	Leaves of the plant from different localities in Palestine were extracted by various methods. The results show clear variations[104]
	Anticancer (prostate), fortified extract	Aqueous extract with/without isovanillin, linolenic acid and β-sitosterol was tested for anticancer activity. These compounds have significantly fortified the activity[105]
	Endangered plant	This mini-review presents the latest publications of the medicinal activities of the plant and classify it as endangered. This might be true in some regions, however, this is not the case in Israel[106]
	Enzyme inhibition	Ethanolic and aqueous extracts were tested for *in vivo* and *in vitro* enzyme inhibition. Both were active as inhibitors of gastrointestinal enzymes involved in carbohydrate and lipid digestion and absorption[64]
	Antioxidant activity	Methanolic and aqueous extracts were prepared, analyzed for phenolic content and tested for antioxidant activity. Methanolic extract was more active[65]

Two of the major (>5%) compounds are both chemically and biologically interesting. These are the structurally isomeric alcohols *T*-cadinol (8.9% in the essential oil of *A. Conophalloides*) and *T*-muurolol (24.4%). Their structures are shown in **Figure 3**.

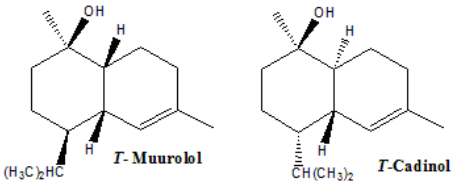

Figure 3. Structures of *T*-cadinol and *T*-muurolol

These compounds have many biological activities such as antibacterial of *T*-cadinol.[116] They are present in relatively high concentrations, and it might be useful to try to isolate them from other subspecies of *Arum*.

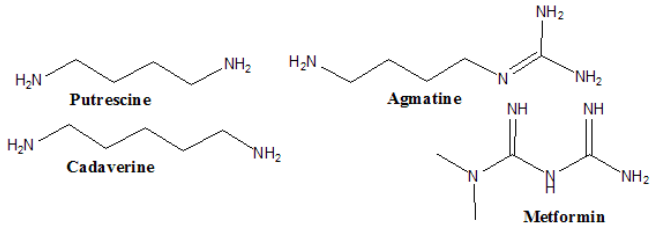

Figure 4. Selected flowering amines of *A. italicum* and metformin

A. italicum produces a wide variety of amines during the flowering season.[56] Some of these amines are very interesting regarding the number of nitrogen atoms that they contain. In **Figure 4**, the structures of three of these amines are shown (with metformin).

The structures of agmatine and metformin are relatively close. Metformin is very well known synthetic antidiabetic drug, and the great medicinal potential and activities of agmatine are being studied, including antidiabetic activity,[117], but this research must be expanded.

Antibacterial and antimicrobial activities are tested for almost every studied medicinal plant. In the case of *Arum* subspecies, some were reported, and these reports are not consistent. Even after taking into account the different subspecies, various parts of the plants that were extracted and the various solvents that were used, the overall reporting is confusing and even contradicting.[60,61,68,69,83,84,95] For example, M. Obeidat *et al*.[60] reported that they tested four extracts (water, ethanol, methanol and acetone) and found the aqueous extract most active. On the contrary, A. Ucar Turker and her colleagues,[68] used aqueous, ethanolic and methanolic extracts, and the aqueous extract was inactive.

In 1994, M. Della Greca *et al*. isolated and characterized phytosterols and hydroperoxy sterols from *A. italicum*, where some were new.[70] Despite the fact that similar compounds were isolated from other plants and marine animals,[118] and proved to have significant biological activities, a follow-up study was never reported. It is worth trying to find this compound family in other *Arum* subspecies, characterize them and test them for biological activities, especially antimicrobial and antifungal activities. The presence of the peroxy group ensures oxidant activity, while the entire compound is hydrophobic and can penetrate the lipophilic membranes of microbes and fungi. The structures of the new hydroperoxy sterols that were reported by M. Della Greca *et al*. are presented in **Figure 5**.

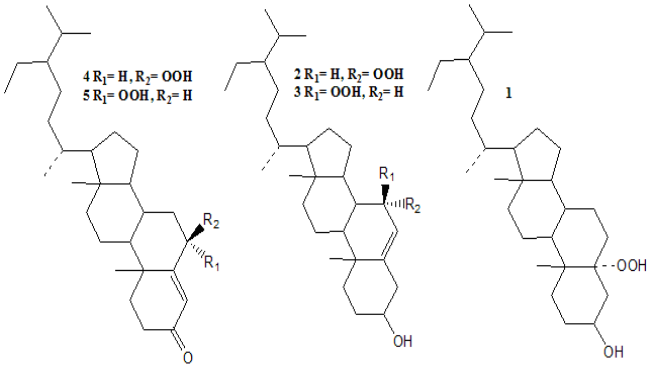

Figure 5. Structures of new hydroperoxy sterols reported in reference 70

Alkaloids are the major compound family in *Arum* subspecies. Their toxic and psychoactive influence affected users of this genus since very ancient times. But the isolation and characterization of these compounds from *Arum* started relatively late.[89] The polyhydroxy alkaloid that was isolated in the same year,[90] provides an interesting starting material for synthetic purposes. Two other new alkaloids were reported later, and they have even simpler structures. *Arum* subspecies can be the natural source for such heterocyclic alkaloids. See **Figure 6**.

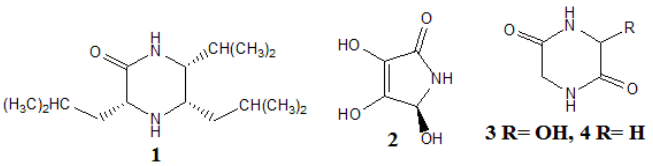

Figure 6. Selected alkaloids isolated from *Arum* subspecies

Reading the papers of M. M. Farid *et al*.[96] of K. I. Ereifej *et al*.[99] reveals some confusion regarding the presence of caffeic acid in *A. palaestinum*. The first reports that it is present, while the second clearly indicated (ND) that it is not. But studying other reports show that this compound is present in *A. palaestinum*, and many of its derivatives.[90,100]

In the same sense, it is not clear why M. M. Farid *et al.*[102] claim that isovitexin was "isolated (by them) for the first time from the studied taxa," while they reported the isolation of the same compound in one of their earlier works.[96]

Conclusions

1) Many subspecies of the genus *Arum* were never studied, which is a very vast field of future potential research.

2) It is important to invest more research in nitrogen containing compounds of *Arum* subspecies. The structures of the known compounds so far (very few ssp.) indicate a high potential for antidiabetic activity, which might result from a single compound or synergy of several compounds.

3) Antibacterial activities of *Arum* subspecies need further studies and organization.

4) Some activities like the antidiabetic potential of *Arum* were hardly investigated. There is an urgent need to expand the research of these activities.

5) Very few attempts were made so far to prepare synthetic modifications of active natural products isolated from *Arum* which could be intensified.

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A SIMPLE, EXPEDITIOUS AND GREEN PROCESS FOR KNOEVENAGEL CONDENSATION OF PYRAZOLE ALDEHYDES

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Knoevenagel condensation of pyrazole aldehydes with malononitrile is selectively carried out using ammonium carbonate as a mild, cheap, efficient and selective catalyst, in aqueous media at ambient temperature under sonication. This method is green and providing an expeditious way for Knoevenagel condensation of pyrazole aldehyde.

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Introduction

Emil Knoevenagel (in 1890) developed a method for the synthesis of substituted alkenes, by the condensation of an aldehyde with active methylene compounds in the presence of base and water. Knoevenagel condensation is typical C-C bond forming reaction in organic synthesis. This reaction is useful to generate a variety of intermediates which are used in the synthesis of pharmaceutical precursors; because of this, Knoevenagel condensation has been extensively studied by researchers. Scientist and academicians are still inventing novel methods and catalysts for Knoevenagel condensation. Several methods have been developed by using the microwave,[1] or ultrasonication,[2] photochemical condensations with fruit extract as a catalyst,[3] solvent free conditions.[4] Recently Franca Bigi et al.,[5] reviewed Knoevenagel reactions in an aqueous medium with and without a catalyst. According to Franca although reaction involves a dehydration step, the reaction can be carried out in water. Following this interpretation, we have carried out the Knoevenagel reaction of pyrazole aldehyde in water but resulted in lower yield due to less solubility of pyrazole aldehyde. Then we have carried out the reaction in the water-ethanol mixture and obtained a high yield of products; these results prompted us to investigate this reaction further.

In literature several methods have been reported for Knoevenagel condensation by using different solvents and homogeneous or heterogeneous catalyst such as, Ti(O-*i*-Pr)$_4$,[6] I_2/K_2CO_3,[7] Ti(O-i-Pr)$_4$/pyridine,[8] calcined egg shells,[9] hydroxyapatite supported $CsCO_3$,[10] amino-functionalized mesoporous silica,[11] mesoporous Ni-Fe hydrotalcite,[12] amino-functionalized mesoporous zirconia,[13] $CaMg(CO_3)_2$,[14] microporous carbon nitride,[15] proline functionalized polyacrylonitrile fibre,[16] sevelamer,[17] basic ionic liquid supported on hydroxylapatite-encapsulated γ-Fe_2O_3 nanocrystallite,[18] Ionic liquids,[19-22] and very recently without catalyst.[23-24] To eliminate or reduce some harsh reaction conditions, harmful and expensive reagents and solvents, we have developed a green method, for Knoevenagel condensation of substituted pyrazole aldehydes with malononitrile in an aqueous medium, using ammonium carbonate as cheap, environmentally friend catalyst.The striking features of reaction are shorter reaction time, ambient reaction temperature, cost effective, simple workup procedure, an aqueous medium (Scheme 1).

Experimental

All chemicals used were of the synthetic grade. The solvents were distilled before use. The progress of the reaction was monitored by TLC using ethyl acetate: n-hexane system. Melting points were recorded by using the open capillary method and are uncorrected. The Ultrasonicator used was made by Cyberlab Ultrasonic Stericleaner model number CB2080 with operation voltage 220 V AC and electric cycle 50/60 Hz. IR spectra were recorded on Shimadzu IR Affinity 1 instrument using KBr discs. H^1 NMR was recorded on BRUKER Avance II 400 NMR Spectrometer using DMSO d_6 as a solvent. The mass was recorded on WATERS, Q-TOF Micro mass (ESI-MS) using methanol as a solvent.

General procedure for the Knoevenagel condensation

In 50 mL round bottom flask pyrazole aldehyde (1 mmol), malononitrile (1 mmol), were taken in 10 ml water- ethanol (1:1) mixture and stirred for 3-5 minutes to mix the reaction mixture; after that ammonium carbonate (20 mol %) was added. The resulting reaction mixture was stirred for 3-20 minutes at reflux temperature, and the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was allowed to cool down to room temperature and then filtered off, washed with water and dried. Similarly, other derivatives were also prepared (Table-2). Similar results were obtained when the reactions were carried out using sonication method (Table 2).

Representative spectral data

2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)malononitrile (3a)

IR (KBr, cm^{-1}): 2942 (Ar-CH), 2225 (CN), 1642, 1533 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):7.49-7.94 (m, 10H,Ar-H), 8.20 (s, 1H, Ar-H pyrazole), 9.21 (s, 1H, Vinyl CH); ESI-MS (m/z): 297(M+1); Molecular Formula:- $C_{19}H_{12}N_4$.

2-((3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-malono-nitrile (3b)

IR (KBr, cm^{-1}): 2940 (Ar-CH), 2218 (CN), 1604, 1525 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):7.49-7.95 (m, 9H,Ar-H), 8.26 (s, 1H, Ar-H pyrazole), 9.22 (s, 1H, Vinyl CH); ESI-MS (m/z): 378(M+1); Molecular Formula:- $C_{19}H_{11}BrN_4$.

2-((3-(2,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methy-lene)malononitrile (3e)

IR (KBr, cm^{-1}): 3154 (Ar-CH), 2239 (CN), 1604, 1525 (C=C);^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):7.43-7.56 (m, 6H,Ar-H), 7.60 (s, 1H, Ar-H pyrazole), 7.78-7.80 (d, 2H,Ar-H), 9.05 (s, 1H, Vinyl CH); ESI-MS (m/z): 365(M+1); Molecular Formula:- $C_{19}H_{10}Cl_2N_4$.

2-((3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-malononitrile (3f)

IR (KBr, cm^{-1}): 3141 (Ar-CH), 2233 (CN), 1587, 1512 (C=C);^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):7.39-7.96 (m, 9H,Ar-H), 8.23 (s, 1H, Ar-H pyrazole), 9.21 (s, 1H, Vinyl CH); ESI-MS (m/z): 315(M+1); Molecular Formula:- $C_{19}H_{11}FN_4$.

2-((3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-malononitrile (3g)

IR (KBr, cm^{-1}): 3411 (OH), 3134 (Ar-CH), 2233 (CN), 1579, 1518 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.93-6.95 (d, 2H, J= 8 Hz, Ar-H), 7.46-7.62 (m, 5H, Ar-H), 7.92-7.94 (d, 2H, J= 8 Hz, Ar-H), 8.16 (s, 1H, Ar-H pyrazole), 9.16 (s, 1H, Vinyl CH), 9.90 (s, 1H, Ar-OH); ESI-MS (m/z): 311(M-1); Molecular Formula:- $C_{19}H_{12}N_4O$.

2-((1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)methylene)-malononitrile (3h)

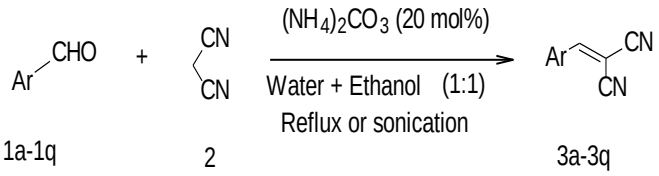
IR (KBr, cm^{-1}): 3133 (Ar-CH), 2230 (CN), 1594, 1521 (C=C);^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):7.47-7.58 (m, 4H,Ar-H), 7.73 (s, 1H, Ar-H pyrazole), 7.81-7.83 (d, 2H, Ar-H), 7.83-7.92 (d, 1H, Ar-H), 8.80-8.85 (m, 2H, Ar-H), 9.10 (s, 1H, Vinyl CH); ESI-MS (m/z): 298(M+1); Molecular Formula:- $C_{18}H_{11}N_5$.

2-(4-(piperidin-1-yl)benzylidene)malononitrile (3q)

IR (KBr, cm^{-1}): 2957 (Ar-CH), 2233 (CN), 1604, 1521(C=C); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):1.64 (q, 6H,CH_2), 3.51 (t, 4H, CH_2), 6.97 (d, 2H, Ar-H, J = 9Hz), 7.82 (d, 2H, Ar-H, J = 9Hz), 7.91 (s, 1H, Vinyl CH); ESI-MS (m/z): 238(M+1); Molecular Formula: -$C_{15}H_{15}N_3$.

Result and Discussions

Owing to the importance of Knoevenagel condensation reaction to synthesise pharmaceutical intermediates, we have developed a green and efficient method, for the synthesis of heterocyclic, substituted alkenes, by reacting pyrazole aldehyde, malononitrile in the presence of ammonium carbonate (20 mol%) in water: ethanol (1:1) mixture at reflux temperature.



To optimize reaction conditions we have performed the reaction of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde with malononitrile and ammonium carbonate which was considered as a standard model reaction. In search of a suitable solvent, we have achieved reaction by using different solvent mixtures and results were summarized in (Table1).

Table 1. The reaction of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde and malononitrile in the presence of 20 % and ammonium carbonate in 10 ml of the solvent system at reflux temperature.

Solvent (mL)	$(NH_4)_2CO_3$, mol %	Time min	Isolated yield, %
DMF	20	30	Nil
Acetonitrile	20	30	Traces
Dioxane	20	30	30
Methanol	20	30	40
Ethanol	20	30	45
DMF-water (1:1)	20	30	Traces
MeCN- H_2O (1:1)	20	30	Traces
Dioxane-Water (1:1)	20	30	25
Ethanol-water (1:1)	--	60	35
Ethanol-water (1:1)	10	60	60
Ethanol-water (1:1)	20	10	92

Water: ethanol (1:1) mixture is proved to be the best solvent system for this reaction. If the reaction was carried out either in water or ethanol; yield of product was decreased due to the higher solubility of either pyrazole aldehyde or ammonium carbonate. The amount of ammonium carbonate has a large influence on the reaction time and yield, the reaction in the absence or presence of 10 % ammonium carbonate only 30 or 60 % yield could be achieved, respectively, and unreacted aldehyde remained back even after 1 h reaction time.

Table 2. Reaction of 1H-pyrazole-4-carbaldehydes and malononitrile in the presence of 20 % and ammonium carbonate in EtOH: H_2O=1: 1 (v/v) system under sonication

No.	R	Reflux temperature		Sonication		Melting points (^{0}C)	
		Time, min	Yield, %[a]	Time, min	Yield,%[a]	Obtained	Reported
3a	1,3-diphenylpyrazol-1-H-4-yl	10	92	08	90	160–162	158-160[1d]
3b	1-phenyl-3-(4-bromophenyl)-pyrazol-1H-4yl	10	91	12	92	193-195	--
3c	1-phenyl-3-(4-chlorophenyl)-pyrazol-1H-4yl	12	80	11	78	212-214	217-219 [1d]
3d	1-phenyl-3-(4-methoxyphenyl)-pyrazol-1H-4yl	15	90	10	85	176-177	176-177 [1d]
3e	1-phenyl-3-(2,4-dichlorophenyl)-pyrazol-1H-4yl	12	86	11	80	175-176	--
3f	1-phenyl-3-(4-fluorophenyl)-pyrazol-1H-4yl	15	80	09	73	217-218	--
3g	1-phenyl-3-(4-hydroxyphenyl)-pyrazol-1H-4yl	20	86	14	84	227-229	--
3h	1-phenyl-3-(3-pyridyl)pyrazol-1H-4yl	15	90	12	86	220-221	--
3i	phenyl	05	90	05	90	83-84	83-84 [4b]
3j	4-chlorophenyl	06	94	04	92	162-163	161 [4b]
3k	4-methoxyphenyl	06	95	04	96	114-115	113-114 [4b]
3l	4-hydroxyphenyl	07	90	05	92	188-189	190 [4b]
3m	4-fluorophenyl	05	92	06	90	121-122	122-124 [4b]
3n	4-bromophenyl	05	90	04	93	152-153	153-155 [4c]
3o	4-dimethylaminophenyl	04	95	02	92	180-181	179-180 [4c]
3p	4-nitrophenyl	03	92	03	91	159-161	161-162 [4c]
3q	4-(1-piperidinyl)phenyl	05	93	04	94	120-122	122-123 [39]

[a]Isolated yield obtained after using 20 mol % ammonium carbonate in 10 ml of water-ethanol (1:1) solvent system.

We have also carried out the same reaction by using sonication to increase yield and reduce the period of reaction. But much difference was not found (Table 2).

Conclusion

The reported method described a simple and fast way for the condensation of pyrazole aldehydes with malononitriles, in 1:1 EtOH: an H_2O solvent mixture in the presence of 20 mol % $(NH_4)_2CO_3$ and under 10 min reflux or sonication.

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LIGHT MICROSCOPIC STUDIES ON THE MORPHOLOGY OF TISSUES EMBEDDED IN EPOXY RESIN

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Keywords: Light optical microscopy, epoxy resin, coronary artria disease (CAD), liver, chronic stress

The best preservation of tissue structures for a long time has been reached by the embedding of tissues into epoxy resins. The aim of the current research is to show the potential of light microscopic studies on the research of blood vessels of tissues embedded and conservated in epoxide resins. Our results demonstrated that preservation of tissue in epoxide resins proved to be a convenient method for theirs studying under light microscope independently of the age and temperature conditions of the sample storage.

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INTRODUCTION

Making stable thin sections of biological tissues with their subsequent staining and analysis using light microscope helps to analyze animalcular texture of the organs.[1-9]

Recently paraffin, celloidin and gelatin pouring of tissues allow to extract histologic specimen applying different ways of staining and to carry out immunohistochemical investigations. At the same time, the quality of tissue structures' preservation remains moderate.[9] The best preservation of tissue structures is being reached at tissues' epoxy injection followed by preparation of semithin sections. Epoxy resins provide optimal tissue morphology at both the light and the electron microscopic level and therefore enable correlative studies on semithin and thin sections from the same tissue block.[6] In the three-dimensional reconstruction of a neuronal structure, it is imperative that ribbons of semithin or ultrathin sections be obtained. Resin-embedded semithin sections display better structural details than paraffin-embedded sections.

The aim of the current research is to show potential for light optical examination of blood vessels of tissues included in epoxide resins for a long-term.

MATERIALS AND METHODS

Reagents: Powdered paraformaldehyde; OsO_4; Sodium cacodylate trihydrate; 96º ethyl alcohol, acetone, Epon 812, Epon Hardener MNA, Epon Hardener DDSA, Epon accelerator DNP-30, uranyl acetate, citrate Na, nitrate Pb, photo plates, AzurII, sodium borate. All reagent used were of analytical grade and purchased from Sigma Chemical Co. (USA).

Biopsy materials of human being myocardium at mitral valve replacement in patients with rheumatic heart disease and coronary artery disease were received in 1997. Biopsy materials of liver were taken while carrying out an investigation of chronic stress in 2010.

All procedures involved human subject were approved by institutional review board/bioethical committee (Erevan State Medical University, RA) conformed to the Legal Aspects of Research Ethics and Science in European Community directive (2001/20/EC). All procedures involving animals were approved by the Institutional Review Board\ Institutional Animal Care and Use Committee (H. Buniatian Institute of Biochemistry, Yerevan, NAS RA) and Ethics Committee of the National Academy of Sciences, the Republic of Armenia, and they were conformed to the European Communities Council's directives (86\609\EC).

Small pieces of tissue have immediately put in a cold mix of paraformaldehyde in a sodium cacodylate buffer and glutaraldehyde for 12 hours at 4 °C with following post fixation in 1% OsO_4 solution for 2, then dehydration in ascending series of spirits; saturation in a mixture of acetone and Epon resins of different proportions to make gelatinous capsules were performed.

Observation under a light microscope: Semifine (semi-thin) epoxy sections with 1 μm thickness were made using LKB (Swedish) and Reichert (Austria) tools and the obtained semithin epoxy sections stained with Azur 2 and studied under a light microscope supplied with 40 x10 ocular lens.

RESULTS

The research of the biopsy material (right auricle atrial of myocardium) and the experimental material (liver) had been carried out. The material had been collected in different years. So biopsy materials of human myocardium were received in 1997 during mitral valve replacement in patients with rheumatic heart disease and coronary artery disease.

Biopsy material of liver was received in 2010 during the investigation of chronic stress. Sampled material was poured into epoxide resins by standard method for transmission electron microscopy. It must be mentioned that for the last years the collected material had been stored at different temperature conditions as low so high.

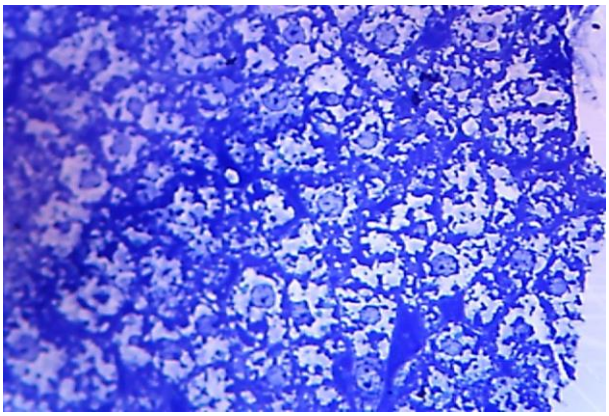

Figure 1. Liver tissue at chronic stress. X 400

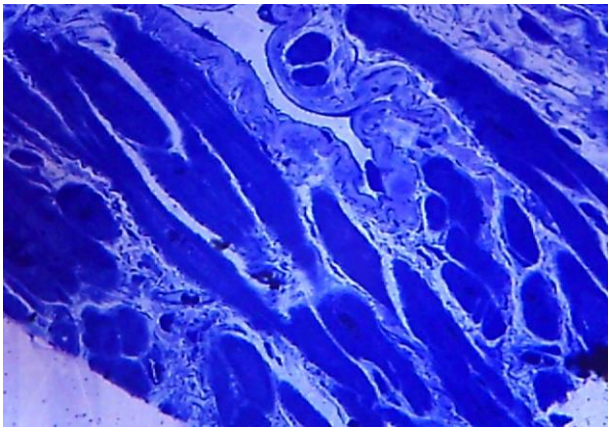

Figure 2. Heart tissue at coronary artery disease. X400

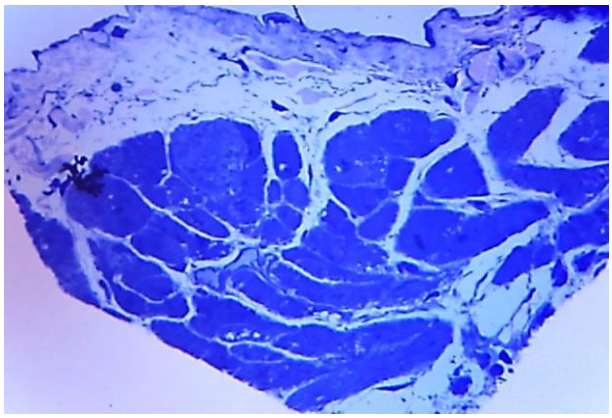

Figure 3. Heart tissue at a rheumatic disease. X 400

The investigation of those materials poured into epoxide resins in different years was carried out. It was necessary to check up how our invention worked[7] on material collected years ago. Nowadays the research on semithin epoxide sections of biological material embedded in epoxide resins provokes more and more interest as it allows seeing thinner morphologic picture under a light microscope. Azur II used by us with sodium borate shows quite interesting results in carrying out current research in diagnosing work on blood vessels in biopsy as it can be seen in Figure 1, 2 and 3.

That pointed out that the current method of investigation allows diagnosing micro circulatory changes as in pathology of a human being so during experimental researches irrespective of the years the material had been collected and the temperature conditions kept at its storing.

DISCUSSION

TEM-examination of ultrathin sections is usually a prerequisite for the researchers working with semi thin epoxide sections of epoxy injected tissue samples.

Semifine epoxide sections are stained in a standard way by Toluidine blue, Methylene blue, AZURE II in different combination. It should be noted that AZURE II almost is not used separately for staining.[2,3] The most common stain used in the electron microscopy lab for thick sections is toluidine blue.[1,4,8,10,11] Unfortunately its general lack of polycromasia makes it unsuitable for photomicrography. Much better results are obtained for general work as well as for photomicrography (especially with the use of filters for the black and white film) with the methylene blue, azure II combinations.[5] They are prepared in advance and most EM-Labs only stained by Toluidine blue because only an approximate morphological information on the area to be sectioned for ultrathin is sufficient.

Toluidine blue is indeed commonly used for stain semithin sections to show the general structure of the cell. Methylene blue-azure is an alternative.[7,9,11,12]

In our research, we used only AZURE II 1% solution prepared on 1% sodium borate by our staining method which turned out to be quite applicable for analyzing blood vessels. Our results show to the well preservation of tissue in epoxide resins for its studying under light microscope irrespective of the time factor and temperature conditions' storage.

CONCLUSION

Collected in different years biological material with shelf life from 18 to 5 years was useful for purposes of light optical research of microvessels on semithin epoxide sections.

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ETHYL LINALOOL AND DIETHYL PHTHALATE FROM PYCNANTHUS ANGOLENSIS (WELW.) WARB.

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Keywords: Pycnanthus angolensis; fraction; chromatography; isolates; antimicrobial effect.

Pycnanthus angolensis (Welw.) Warb. (Family; Myristiceae) leaves were extracted cold with 50 % ethanol and the obtained aqueous crude extract partitioned with ethyl acetate. Furthermore, the ethyl acetate fraction was subjected to silica gel column chromatography and the isolated chemical compounds tested for antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. The identities of two isolates have been revealed to be 3-ethoxy-3,7-dimethyl-1, 6-octadiene (ethyl linalool) and diethyl phthalate (1,2-benzenedicarboxylic acid diethyl ester) using the MS and IR spectral techniques. Both compounds showed strong bacteriostatic action against *E. coli* but were inactive against *S. aureus* and *C. albicans*.

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INTRODUCTION

Pycnanthus angolensis (Welw.) Warb**.** syn. *P. kombo* (known as African or wild or false nutmeg) was originally native to the forest zones of West and Central Africa but now cultivated in and around the world.[1] Different preparations of the plant are employed in diverse African folklores to treat chest infections, diabetes, lumbago, wounds, arthritis, anaemia, mouth-thrush, malaria, leprosy, toothache, infertility, sexually transmitted disease and skin-fungal infections.[2-4] The larvicidal and antitumor potentials of the plant have been studied [5-6] while reports of isolation of flavonoids and terpenes from the bark and roots and quinones from the leaves abound.[7] This present investigation aimed at isolating the compound(s) from the ethyl acetate fraction which demonstrated the highest antimicrobial activity in a previous bioactivity-guided fractionation study of the plant.[8] In addition, the compounds so obtained will be screened for antimicrobial activity with the aim of confirming or disproving the claims highlighted in traditional medicine especially for the treatment/management of bacterial infections.

MATERIALS AND METHODS

The fresh leaves of *P. angolensis* were collected around April, 2016 within the precinct of University of Uyo, Akwa Ibom State, Nigeria. The plant had previously been identified in a study. [8] Immediately after collection, the plant was dried in a laboratory oven (Gallenkamp, England) at 40 ^{0}C for 48 h and the resultant material powdered on an electric mill (Uniscope, England).

Extraction and isolation

The dried powder (1.1 kg) was exhaustively extracted with 50 % EtOH (3 x 5L) at room temperature (27± 2 ^{0}C) for 72 h. The resultant crude extract mixture was filtered, concentrated *in vacuo* on a rotary evaporator. 250 g of dried crude extract was obtained and then stored in a desiccator prior to further use. Consequently, 15 g of the extract was partitioned using H_2O: EtOAc (8 x 200 mL). The combined ethyl acetate fractions were evaporated to dryness to give a brown solid residue. Hence, 1.2 g of the fraction was chromatographed on a silica gel 254 column (Pyrex, USA; 10 g pre-swollen in 100 % toluene; 2 g concentration zone + 8 g separation zone; 13.6 x 4 cm) and eluted with a gradient of 20 % $(CH_3)_2CO$: toluene (48 mL), 30 % $(CH_3)_2CO$: toluene (48 mL), 40 % $(CH_3)_2CO$: toluene (48 mL), 50 % $(CH_3)_2CO$: toluene (48 mL), 60 % $(CH_3)_2CO$: toluene (48 mL) 70 % $(CH_3)_2CO$: toluene (48 mL) and 80 % $(CH_3)_2$ CO: toluene (48 mL). Fractions of 8 mL each were collected and monitored on silica plates in $(CH_3)_2CO$: toluene: H_2O (10:20:1) using $FeCl_3/CH_3OH$ and vanillin-H_2SO_4 as spray reagents.

Hence, fractions with similar TLC characteristics (R_f values, reaction with vanillin-H_2SO_4 spray) were bulked and dried. Five sub-fractions coded NG-1, NG-2, NG-3, NG-4 and NG-5 were obtained. Further TLC examinations of these sub-fractions in $(CH_3)_2CO$: toluene: H_2O (10: 20: 1) and $(CH_3)_2CO$: EtOAc (35: 65) indicated a single spot in **NG-2** (pale yellow compound; R_f (0.53); 62 mg) while the others showed multi-component TLC profiles. Attempts were made to clean up the semi-pure residues separately NG-1, NG-3, NG-4 and NG-5 on a short silica gel 254 column (7.8 x 4 cm) using 50 % $(CH_3)_2CO$: toluene (48 mL). However, only NG-4 furnished a single spot. Hence, **NG-4c** was isolated (off-white compound; R_f (0.24); 36 mg). The refractive indices and optical rotation were obtained using WAY-15 Abbe refractometer (England) and ADP-220 Bellingham Stanley polarimeter (England) respectively. Refractive indices and optical rotation were measured at the wavelength (λ) of Na^D line (589.3 nm) and 20 °C.

Antimicrobial tests

The microorganisms used in this study, namely; *Staphylococcus aureus* (ATCC 21824), *Escherichia coli* (ATCC 2353) and *Candida albicans* (NCYC 106) were clinically isolated from specimens of diarrheal stool, abscesses, necrotizing fasciitis, urine and wounds obtained from the Medical Laboratory, University of Uyo Health Centre, Uyo. The clinical isolates were collected in sterile bottles, identified and typed by convectional biochemical tests.[9-10] These clinical microbes were then refrigerated at -5 ^{0}C. The agar plates used were prepared by adhering to the manufacturer's instructions. The media and plates were sterilized in an autoclave at 121°C for 15 min.

The hole-in-plate agar diffusion method was used observing standard procedures for Nutrient Agar-CM003, Mueller-Hinton-CM037 (Biotech Limited, Ipswich, England) and Sabouraud Dextrose Agar (Biomark, India) in respect of bacteria and fungus respectively. The inoculum of each micro-organism was introduced into each petri-dish (Pyrex, England). Cylindrical plugs were removed from the agar plates using a sterile cork borer (Simax, India) to produce wells with a diameter of approximately 5 millimetres. The wells were equidistant from each other and the edge of the plate.[11-12] Concentrations of 20 mg mL^{-1} of crude extract, 10 mg mL^{-1} of ethyl acetate fraction, 2 mg mL^{-1} of **NG-2** and **NG-4c** were introduced into the wells. Also, different concentrations of 10 µg mL^{-1} Streptomycin (Orange Drugs, Nigeria), 1 mg mL^{-1} of nystatin (Gemini Drugs, Nigeria) and deionized water were introduced into separate wells as positive and negative controls respectively.[5,13-15] The experiments were carried out in triplicates. The plates were labelled on the underside and left at room temperature for 2 h to allow for diffusion. The plates were then incubated at 37 ± 2 ^{0}C for 24 to 48 h. Zones of inhibition were measured in millimetres (mm) with the aid of a ruler.

RESULTS AND DISCUSSION

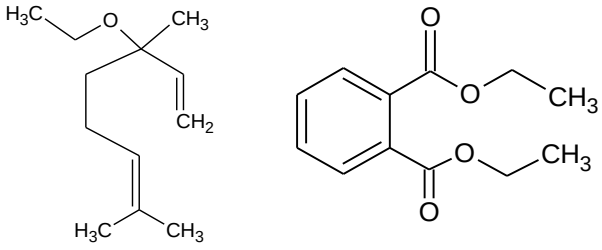
The two isolated compounds were identified as 3-ethoxy-3,7-dimethyl-1,6-octadiene (ethyl linalool) and diethyl phthalate (1,2-benzenedicarboxylic acid diethyl ester) respectively (Figure 1**)**. The refractive indices of **NG-2** and **NG-4c** were found to be 1.4009 and 1.5006 respectively. These values are consistent with the literature values (1.4006 and 1.5002 given for ethyl linalool and diethyl phthalate respectively.

Structural elucidation

The mass spectra of the compounds were obtained on Kratos MS 80 (Germany) while the infra-red analyses were done on Shimadzu FTIR 8400S (Japan).

NG-2: $C_{12}H_{22}O$; pale yellow compound; R_f (0.53); $[\alpha]_D^{20}$ (+3º); $[n]_D^{20}$ (1.4009); MS [ES+-MS] m/z (relative intensity): 182 $[M]^+$ (0.64 %), 180 $[M-2H]^+$ (0.70 %), 166 $[M-CH_3-1H]^+$ (4.05 %), 150 $[M-2CH_3-2H]$+ (3.30 %), 137 $[M-3CH_3]$+ (3.20 %), 121 $[M-OC_2H_5-CH_3-1]$+ (41.01 %), 107 $[M-OC_2H_5-2CH_3]$+ (8.04 %), 96 $[M-OC_2H_5-3CH_3+4]$+ (51.30 %), 71 $[M-OC_2H_5-3CH_3-21]$+ (90.13 %), 69 $[M-OC_2H_5-3CH_3-23]$+ (30.07 %) and 43 $[M-M+OC_2H_5-2]$+(100.00 %); IR [FTIR] cm-1: 923, 912, 876 (alkyl substitution), 1652 (acyclic -C=C) and 1087 (-C-O).

NG-4c: $C_{12}H_{14}O_4$ (off-white compound; R_f (0.24); $[\alpha]_D^{20}$ (0º); $[n]_D^{20}$ (1.5006); MS [ES+-MS] m/z (relative intensity): 222 [M]+ (1.42 %), 194 $[M-C_2H_5+1]$+ (3.30 %), 177 $[M-OC_2H_5]$+ (37.73 %), 164 $[M-OC_2H_5-CH_3-2]$+ (4.06 %), 149 $[M-OC_2H_5-2CH_3+2]$+ (100 %) 132 $[M-2OC_2H_5]$+ (5.26 %), 121 $[M-2OC_2H_5-11]$+ (6.08 %), 105 $[M-2OC_2H_5-CO-1]$+ (7.31 %), 93 $[M-2OC_2H_5-CO-11]$+ (7.50 %), 78 $[M-2OC_2H_5-2CO+2]$+ (23.05 %), 65 $[M-2OC_2H_5-2CO-11]$+ (18.26 %) and 50 $[M-2OC_2H_5-2CO-25]$+ (20.15 %); IR [FTIR] cm-1: 932, (alkyl substitution), 1072 (-C-O-C), (1602) Ar (-C=C) and 1721 (-C=O).



NG-2 **NG-4c**

Figure 1. 3-ethoxy-3, 7-dimethyl-1, 6-octadiene (ethyl linalool, **NG-2**) and 1, 2-benzenedicarboxylic acid diethyl ester (diethyl phthalate, **NG-4c**).

In the mass spectrum of ethyl linalool, the molecular peak could be assigned easily at m/z 182 (0.64 %) while fragments at 166 (4.05 %), 150 (3.30 %) and 137 (3.20 %) represent the excision of methyl group(s) from $[M]^+$. Furthermore, ions at 121 (41.01%), 107 (8.04 %), 96 (51.30 %), 71 (90.13 %) and 69 (30.07 %) correspond to the losses of ethoxy and methyl groups from **NG-2.** The peak at 43 (100 %) (base peak) indicates the disintegration of the molecule save for an ethoxy group.

The FTIR spectrum of **NG-2** shows absorptions at 1652 and 1087 cm^{-1} indicating acyclic -C=C and -C-O-C (ether linkage) respectively. The compound, ethyl linalool showed an optical rotation of +3° indicating dextrorotation.

Equally, **NG-4c** (diethyl phthalate) showed $[M]^+$at m/z 222 (0.12 %), while ions at 177 (37.73 %), 132 (5.26 %) and 121 (6.08 %) indicate the loss of ethoxy group(s) from the molecule. In addition fragments at 164 (4.06 %) and 149 (100%) (base peak) correspond to the excisions of ethoxy and methyl group(s) from **NG-4c.** Furthermore, ions at 105 (7.31 %), 93 (7.50 %), 78 (23.05 %) 65 (18.26 %) and 50 (20.15 %) show the removal of ethoxy and carbonyl group(s) from $[M]^+$.

The IR spectrum of **NG-4c** shows diagnostic stretchings at 1721, 1602 and 1072 cm^{-1} representing (-C=O), Ar (-C=C) and-C-O-C (ether linkage) functional groups respectively.

Table 1. Results of antimicrobial screening of crude extract, ethyl acetate fraction, **NG-2** (ethyl linalool) and **NG-4c** (diethyl phthalate) at different concentrations on test microbes in deionized water

Species	CE, 20 mgmL^{-1}	ET, 10 mgmL^{-1}	NG-2, 2mg mL^{-1}	NG-4c, 2 mg mL^{-1}	H_2O	SP, 10 µg mL^{-1}	NY, 1mg mL^{-1}
S. aureus	5	5	5	5	5	26	5
E. coli	5	5	17	15	5	31	5
C. albicans	5	5	5	5	5	5	29

Key: The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +5) mm; **CE** = Crude ethanolic extract; ET = Ethyl acetate fraction; S**P** =Streptomycin; **NY** =Nystatin;**NG-2**=3-ethoxy-3,7-dimethyl-1,6-octadiene (ethyl linalool);**NG-4c =** Diethyl phthalate (1,2-benzenedicarboxylic acid diethyl ester);**NCTC** - National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9, UK.**NCYC**- National Collection of Yeast Cultures, UK. **ATCC**- American Type Culture Collection**,** Washington, DC. *S. aureus* (ATCC 21824), *E. coli* (ATCC 23523), *C. albicans* (NCYC 106).

Antimicrobial screening

The spectrum of microbes employed in the sensitivity tests was narrow, encompassing one each of gram positive (*S. aureus*) and gram negative (*E. coli*) bacterial strains and a fungus (*C. albicans*). The results displayed in **Table** 1 show that the crude extract, ethyl acetate fraction, **NG-2**, and **NG-4c** were inactive against *S. aureus* and *C. albicans.* However, the two compounds were remarkably bacteriostatic against *E. coli.* This result was unexpected because gram-negative bacteria are well known for their unique resistance to antimicrobial agents. This resistance is believed to be due to the nature of the cell envelope of these organisms which unlike gram-positive organisms possess a sophisticated three-layered envelope which does not allow permeation of external agents. Also, both compounds demonstrated no antifungal activity against *C. albicans.* This particular observation was to be expected because of fungal strains especially *Candida spp*. limit the permeation of substances because of their integral structures which are pleomorphic and facultative in nature hence, resembling those of higher plants.[16] It is instructive to mention that derivatization studies are currently on-going in our laboratories with the aim of improving on the observed activity.

CONCLUSION

The isolation of the two compounds is being reported for the first time from the ethyl acetate fraction of the plant. Hence, ethyl linalool and diethyl phthalate are expected to serve as chemotaxonomic markers for this species and the genus, *Pycnanthus* in general. Furthermore, the results of the antimicrobial sensitivity tests lend some credence to the use of this plant especially in the treatment or management of the bacterial disease.

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ON DYNAMICS OF ROTATIONAL MOTIONS IN MACRO- AND MICROSYSTEMS

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Keywords: orbital and rotational motion; direct and reverse rotation; planets; rotation angles

Structural-dynamic processes in macro- and microsystems, including the specifics of formation of direct or reverse rotation of the planets are explained based on the previously proposed method to evaluate the corpuscular-wave mechanism. Equations of dependence of rotational and orbital motions of planets are given, their rotation angles are calculated. Wave principles of direct and reverse rotation of planets are also established.

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Introduction

The world around us is in a constant motion. Functionally interconnected main types of mechanical motion (translational, rotational and oscillatory) determine the dynamic stability of systems. Vast theoretical and experimental experience in physical and mathematical properties of simple and complex compounds and principles of their self-organization at different scale levels of such conformation has been gained till now. But the problem of establishing the most general regularities of these processes is topical. "However the science is still far from making it happen in the general form".[1] Thus, applying the entire set of analytical and qualitative methods, the celestial mechanics provides the solution for many problems on the motion of solids, for example.[2,3] But some other issues of celestial mechanics require further discussion, for instance, the functional dependence of rotational and orbital motion of planets, as well as the initial principles of forming the direct and reverse motion of planets. Therefore in this paper we attempt to investigate such problematic issues with the help of conception of corpuscular-wave dualism proposed earlier.[4]

Initial criteria[4]

1. In the systems in which the interaction proceeds along the potential gradient (positive work), the resultant potential energy is found based on the principle of adding reciprocals of corresponding energies of subsystems. Similarly, the reduced mass for the relative motion of the isolated systems of two particles is calculated.

2. In the systems in which the interactions proceed against the potential gradient (negative performance) the algebraic addition of their masses, as well as the corresponding energies of subsystems is performed (similar to Hamiltonian).

3. Two principles of adding energy characteristics of structural interactions can be transformed onto the corpuscular-wave dualism processes.

Corpuscular processes flow in all interactions along the potential gradient, and wave dualism corresponds to the interactions against the potential gradient.

4. Act of quantum action expressed via Plank's constant is narrowed to the energy equilibrium-exchange redistribution between the corpuscular and wave processes.

5. Phase difference of electric and magnetic oscillations in electromagnetic wave is $\pi/2$. Applying $(2/\pi)^2$ as the proportionality coefficient, we have the equation for Plank's constant with the accuracy close to the accuracy of the initial data:

$$h = \left(\frac{4}{\pi^2} + a_0 \right) P_e \frac{\varepsilon}{\mu}$$

where

a_0 =0.0023293 – the factor of experimental quantum correction to spin g_s

ε – electric constant,

μ – magnetic constant.

Here

$$P_e = wr,$$

where

w – energy of a free electron,

r – its classic radius.

6. It is assumed that during the rotational-translation motion of an electron, the energies are redistributed in the system "particle-wave" that is demonstrated via the angular vector of such motion (winding angle) – Θ.

This angular vector of electron motion is quantized by an integer number through the tangent square of this angle: $tg^2\varphi_r = 2$; $tg^2 60^o = 3$; $tg^2 45^o = 1$, where $\varphi_r = 54.73^0$ – a so-called "geodesic angle", which is widely spread in engineering, for example, in spaceship production.

The quantum functions of square tangent k = 1, 2, 3 numerically determine the ratios of two triangle legs, whose values characterize energy dependencies via axial and circumferential stresses in the system with corpuscular and wave processes.

7. In quantum mechanics the ratio between the particle magnetic moment and its mechanical moment is the magnetomechanic ratio – g. At the same time, $g_s = 2$, if the electron magnetic moment is conditioned only by the spin component and $g = 1$, if it is produced by the electron orbital motion. Their ratio $g_s/g = 2$ that, the same way as $tg^2\varphi_r = 2$, characterizes the corresponding corpuscular-wave dependencies in this approach.

Equation of dependence of rotational and orbital motion of planets

The foregoing principles of corpuscular-wave mechanism give the possibility to consider from the unified positions many structural and dynamic processes, different in nature and scale. For example, the characteristic of spin-orbital interaction – fine structure constant $\alpha = r/\lambda$, where r – electron classic radius, λ – its Compton wavelength.

Formally, but similarly: interaction force of two long conductors with the current proportionally to the ratio $l/2\pi r$, where l – length of conductors, r – distance between them.

And the number 2π widely used in physical regularities, equals the circumference ratio to its radius: $2\pi = l/R$.

In these examples, as in many other, this approach allows evaluating structural interactions based on the ratios of corpuscular and wave spatial-energy parameters in each action. Obviously, such principles are also demonstrated in Kepler's third law, which can be given as follows:[3]

$$Gm = 4\pi^2 \frac{a^3}{T^2} \qquad (1)$$

where:

G – gravitational constant,

m – planet mass,

a – distance to barycenter (system mass center),

T – planet revolution period.

Entering the relative mass $m_0 = m/m_e$ (where m_e=Earth mass)we can have:

$$\frac{Gm_e}{4\pi^2} = \frac{a^3}{T^2 m_0} \qquad (2)$$

therefore $a^3/T^2 m_0$ = const or $a/(T^2)^{1/3} m_0^{1/3}$ = const.

Since the masses of planets are rather small in comparison with their distance to the sun, then at the first approximation they can be considered as mathematical points and the equation of mathematical pendulum period can be applied to them:

$$T^2 = 4\pi^2 \frac{l}{g} \qquad (3)$$

where g – free fall acceleration.

The average radius of the planet orbital motion – R can be taken as the pendulum length – l. Then, similarly to the foregoing dependencies, we can introduce the planet radius r into the numerator of formula (2) and then we have:

$$\gamma = \frac{r}{\left(\frac{4\pi^2}{g} R m_0\right)^{1/3}} \qquad (4)$$

This expression in the units $m/s^{2/3}$ satisfies the principle of corpuscular-wave mechanism for space macrostructures. But in Kepler's third law only the orbital motion was considered, but in this case – two motions, each of which has its own wave part. Therefore, the interference of coherent waves occurs.

Similar to the foregoing examples, the coherence can be considered as the ratio of the difference of the travel path to the length of the coherent wave Δ/λ. The interference principle is most easily performed for liquid-gaseous planets (planets in Jupiter system) as shown in Table 1:

$$\beta_+ = 2n \frac{\gamma}{2} \qquad (5)$$

-boosting of oscillations during the direct rotation of planets.

$$\beta_- = (2n+1) \frac{\gamma}{2} \qquad (6)$$

-damping of oscillations during the reverse rotation of planets. Here n – integer number.

The intensity of wave propagation depends on the medium density and its distribution in the planet volume.

The value characterizing the planet density increase towards its center is called "dimensionless moment of inertia"(I^*). The ratio of average values of I^* for solid and liquid-gaseous planets based on different data[3,5,6] is within 1.4 – 1.45 as demonstrated in Table 2.

Table 1. Characteristics of rotational and orbital motion of planets

Planet	$r\cdot10^6$, m	$R\cdot10^9$, m	γ, m/s$^{2/3}$	Rotation	Calculation formula for β	n	β, m/s$^{2/3}$	δ=β/β$_0$	Θ°$_1$	Θ°$_2$	Θ° [3,5,6]
Mercury	2.4397	57.9	1039.7	+	$n^{1/2}\gamma/2$	2	735.2	0.9735		86.6	87.0
Venus	6.0515	108.2	855.17	-	$(2n+1)^{1/2}\gamma/2$	1	740.6	0.9810		87.2	87.0
Earth	6.3780	149.6	755.2	+	$2n^{1/2}\gamma/2$	1	755.2	1	66.56		66.556
Mars	3.3970	227.9	734.7	+	$2n^{1/2}\gamma/2$	1	734.7	0.9728	64.75		64.8
Jupiter	71.492	778.6	715.8	+	$2n\gamma/2$	1	715.8	0.94781/2		86.6	86.9
Saturn	60.268	1433.7	735.9	+	$2n\gamma/2$	1	735.9	0.9744	64.85		64.3
Uranus	25.596	2870.4	463.6	-	$(2n+1)\gamma/2$	1	696.8	0.9227		82.07	82.0
Neptun	24.764	4491.1	365.49	+	$2n\gamma/2$	2	730.98	0.967932	62.5		61.68
Pluto	1.1510	5868.9	295.55	-	$(2n+1)\gamma/2$	2	738.75 ‹β›=732.5	0.9782			

Table 2. Ratio of dimensionless moments of inertia of solid and liquid-gaseous planets[3,5]

Planet	Mercury	Venus	Earth	Mars	‹I^*_S›
I^*_S	0.324	0.333	0.33076	0.377	0.341
Planet	**Jupiter**	**Saturn**	**Uranus**	**Neptune**	**‹I^*_{LG}›**
I^*_{LG}	0.20	0.22	0.23	0.29	0.235

The average value I^*_S / I^*_{LG}=1.451

Such property for solid planets is taken into account in Table 1 and equations (5, 6) by introducing the values n/2 and $(2n+1)^{1/2}$. Such approach also refers to Mercury as it is the nearest to the sun, to its liquid-gaseous structure.

In general, the application of corpuscular-wave mechanism to space macrosystems explains the specifics of formation of direct or reverse rotation of the planets.

Rotation angles of planets

In physical sense, the parameter β characterizes the motion difference of interfering waves, and γ – wavelength. The average value of β = 732.5 m/s$^{2/3}$ with the deviation of most of the planets under 2 % (apart from Uranus).

The equation $tg^2\Theta=k$ was used to evaluate quantum transitions in atoms.[4,7] The squares and cubes of initial parameters are applied in Kepler's equation and other regularities of space macrosystems. In this approach, as the calculations demonstrated, the semi-empirical equation is performed:

$$\beta = (tg^2\Theta)^3 \qquad (7)$$

where Θ – rotation angle of planets.

For Earth β = 755.2 and based on equation (7) Θ_0=66.455°. For more accurate calculation, taking into account some analogy of macro- and microprocesses, we use, as before, the experimental quantum correction in the form of a_0 = 1.0023293 following the equation:

$$\Theta_1 = a_0\Theta_0 \qquad (8)$$

The calculation by equations for Earth (7,8) gives the value Θ_1=66.560º with the deviation from the experimental value by 0.007 %. The sun has the same value of the rotation angle.

As applicable to the rest of the planets, the value δ=β/β$_0$ (where β_0 – β value for Earth) is introduced in equation (8) based on the following equations:

$$\Theta_1 = a_0\delta\Theta_0 \quad \text{or} \quad \Theta_1 = a_0\frac{\beta}{\beta_0}\Theta_0 \qquad (9,9a)$$

$$\Theta_2 = \frac{4}{3}a_0\delta\Theta_0 \quad \text{or} \quad \Theta_2 = \frac{4}{3}a_0\frac{\beta}{\beta_0}\Theta_0 \qquad (10,10a)$$

The formulas (9 and 9a) are performed for the planets, whose rotation angle is less than Earth's one. For the other planets the formulas (10 and 10a) are performed. Those are the planets, which are in the beginning of the planet subsystem by the value of the dimensionless moment of inertia (Mercury and Jupiter), as well as the planets with the reverse rotation (Venus and Uranus). The calculation results are given in Table 1.

The coefficient 4/3 has been applied before for the comparative evaluation of quantum transitions with different complexity types.[4] In this research the average ratio of the angles by the experimental data[3,5,6] given in Table 3 also has the value 1.336 ≈4/3.

Table 3. Ratio of rotation angles of planets by [3, 5, 6]

Planet	Mercury	Venus	Earth	Mars	‹Θ_2›
Θ_2	87.00	87.00	86.90	82.00	**85.73**
Planet	**Jupiter**	**Saturn**	**Uranus**	**Neptune**	**‹Θ_1›**
Θ_1	66.556	64.80	64.30	61.00	**64.16**

The average value Θ_2/Θ_1 = 1.336 ≈4/3

For the value δ the influence of the medium density distribution is taken into account via the transition factor from one distribution level to another. Since for Jupiter in Table 2 the value I^* is less in comparison with Earth's I^* in 1.45 times, therefore, in the calculations $\delta = 0.9478^{1/2}$. On the contrary with Jupiter, for Neptune I^* increases in 1.45 times, therefore, in the calculations $\delta = 0.96793^2$. All the calculation results are in good according with the experimental data.

Conclusions

1. Semi-empirical equations of the dependence of rotational and orbital motion of planets are obtained.

2. Many structural-dynamic processes in macro- and microsystems, including the specifics of formation of direct or reverse rotation of the planets are explained based on the previously proposed method to evaluate the corpuscular-wave mechanism.

3. The given calculations of rotation angles of the planets are within the accuracy of the experimental data.

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NOVEL [(3-NITRO-2-OXO-2H-CHROMEN-4-YLAMINO)-PHENYL]PHENYL-AZETIDIN-2-ONES AND THEIR ANTIMICROBIAL ACTIVITY

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Keywords: azetidin-2-one, 3-nitrobenzopyran-2-one; condensation; cyclization; antmicrobial citvity.

Synthesis of new azetidin-2-ones on the basis of 4-aminophenyl-3-nitrobenzopyran-2-one is reported. By condensation reaction of 4-chloro-3-nitrobenzopyran-2-one **2** and phenylenediamine, 4-(4-aminophenylamino)-3-nitrochromen-2-one **3** is synthesized in high yield. Catalytic condensation of product **3** and benzaldehyde, salicylaldehyde and 3-nitrobenzaldehyde, afforded novel derivatives of 4-[4-(benzylideneamino)phenylamino]-3-nitrobenzopyran-2-one, **4a-4c**. In the following series of reactions, by cyclization of the products **4a-4c** with acetyl chloride, corresponding substituted azetidinones **5a-5c** are synthesized. Structural characterization of the synthesized products is done on the basis of spectrometric data. Compounds of series **4a-4c** and **5a-5c** are examined for their antibacterial activity against *S. aureus*, *E. coli*, and *Klebsiella*. Antibacterial activity is examined by measuring the inhibition zones around the disks marked with the corresponding product solutions in DMF concentration 2 mg mL^{-1}, 4 mg mL^{-1}, and 6 mg mL^{-1}. Compounds of series **4** have shown moderate antibacterial activity against these microorganisms, whereas compounds of series **5** have shown significant activity. The impact of substitutions in antimicrobial activity is also explored.

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Introduction

Coumarin derivatives are an important class of compounds and are well-known for their biological activity. Many of heterocyclic derivatives on the basis 2H[1]-benzopyran-2-one play an important role in various life processes, and they are found as an ingredient in the plant world. Many such derivatives exhibit various biological activities,[1] such as anticoagulant,[2] antimicrobial,[3] antibacterial,[4, 5] antifungal[6] and antimalarial.[7] Some of coumarin analogs also exhibited antioxidant,[8,9] anti-tubercular[10] and anticonvulsant activity.[11] It was reported that a significant number of substituted derivatives of benzopyran-2-one also show, sedative,[12] analgesic and anti-inflammatory,[13] anti-HIV,[14] and hepatoprotective activity.[15] According to that, many of them have found widespread usage in pharmacies.[16] On the other hand, azetidin-2-one derivatives reported having a wide range of biological activities,[17] including those antimicrobial,[18] anti-fungal,[19] anti-convulsant,[20] and anti-HIV activity.[21] The biological activity of these derivatives is conditioned by their structure, so the presence of different substituents on the benzopyrone ring indicates their impact on the type and potency of biological activity. Despite continuous efforts, the relationship between structure and biological activity of these derivatives so far has not yet been sufficiently clarified. Extraordinary biologically importance of such derivatives on the basis of thiazolidine-4-one has generated a constant interest for their synthesis and research. In continuation of our previous studies and attempt to synthesize the new derivates,[22, 23] in this paper we had intended to synthesize some new amino-phenylamino)-3-nitro-chromen-2-ones and substituted thiazolidyne-4-ones with benzopyran-2-one moiety, which could serve as pro-pharmaceutical products.

Methods and materials

The compounds are synthesized using commercial reagents of Aldrich company as precursors under catalytic conditions. Reactions are monitored by TLC using Merck Kieselgel-60 (F-254) as the stationary phase and a mixture of benzene, toluene, glacial acetic acid (v/v/v, 80:10:10) as the mobile phase. The synthesized products are purified by crystallization from methanol and ethanol. Melting points are determined used a paraffin oil bath with an open capillary tube. IR spectra are recorded in KBr discs on Shimadzu 8400xFT-IR spectrometer with 4 cm^{-1} resolution. ^{1}H-NMR and ^{13}C-NMR spectra are recorded in DMSO on UNITYplus-500"NMR 1" spectrometer. Chemical shifts were reported in ppm downfield from TMS as internal standard (δ 0.00). Examination of the antibacterial activity of the synthesized compounds is done using standard discs (*d*=5.0 mm, maximum capacity 10 pg) on the basis of Standard Disc Method. Standard discs are previously impregnated with 2 mg mL^{-1}, 4 mg mL^{-1} and 6 mg mL^{-1} solutions of compounds in -DMF.

Preparation of 4-(4-Amino-phenylamino)-3-nitro-chromen-2-one, 3

4-Chloro-3-nitrobenzopyran-2-one, 2g (9.0 mmol) are dissolved in 6 mL of ethanol, then in small portions was added the mixture containing 0.97g (9.0 mmol) benzene-

1,2-diamine in 5 mL of ethanol and then 2-3 drops of triethylamine was added to the mixture. The content is mixed for 10 min at room temperature, then refluxed for about 90 minutes. After cooling, the mixture is concentrated in the rotary evaporator, and the crystalline product is filtered off under vacuum and washed with 2 × 1 mL of ethanol. The crystalline product is dried and crystallized from methanol, giving) 4-(4-aminophenylamino)-3-nitrocrhromen-2-one, yield was 79.68 %, mp = 240-242 °C. IR (KBr disc, cm^{-1}): 3470.92, 3386.12, 3220.86, 3058.34, 2872.24, 2595.12, 1708.54, 1614.38, 1511.25, 1327.77, 1220.36, 1130.62, 1067.34, 905.94, 757.95.

General procedure for preparation of 4-[4-(benzylidene-amino)-phenylamino]-3-nitrobenzopyran-2-ones, 4(a-c)

Product **3 (**0.3 g, 1.0 mmol) is dissolved in 20 mL of absolute ethanol and to this mixture was added in small portions 1.5 mmol of aromatic aldehyde (benzaldehyde, salicylaldehyde or 3-nitrobenzaldehyde respectively) dissolved in 10 mL of absolute ethanol. Then there was added 2 drops of piperidine as a catalyst and the mixture was mixed for 15 min at room temperature and refluxed for 10 to 12 hours. After cooling, the mixture is concentrated and the crystals are filtered off under reduced pressure, then washed with 2x1 mL of ethanol and dried in the air. crystallization of the products **4a-4c** was conducted from ethanol or methanol.

4-[4-(Benzylidene-amino)-phenylamino]-3-nitro-chromen-2-one, 4a

Yield: 87.26 %, m.p.= 224-226 ºC, IR (KBr disc, cm^{-1}): 3461.45, 3292.27, 3075.24, 2934.85, 1694.15, 1642.76, 1603.07, 1551.15, 1517.34, 1428.72, 1326.42, 1221.34, 1055.46, 902.05, 756.85. ^{1}H-NMR; (δ, ppm) 8.35 (s, 1H, N=C-H), 7.4-7.6 (m, 4H, Ar), 7.2-7.4 (m, 5H, Ar), 7.1 (d, 2H, Ar), 6.5 (d, 2H, Ar),3.9 (1H,NH). ^{13}C-NMR (δ, ppm); 165.4 (C=N), 162.3 (C=O), 160.2, 148.9, 141.5, 131.7, 130.6, 128.3, 127.6, 127.2, 126.8, 126.4, 123.8, 122.1, 115.8, 105.2.

4-{4-[(2-Hydroxy-benzylidene)-amino]-phenylamino}-3-nitro-chromen-2-one, 4b

Yield: 39.88 %, m.p.= 205-207 ºC, IR (KBr disc, cm^{-1}): 3451.94, 3307.35, 3079.24, 2934.74, 2872.64, 2386.73, 1684.15, 1608.97, 1552.51, 1514.34, 1431.54, 1320.49, 1278.62, 1211.64, 1197.59, 1055.44, 903.53, 761.67. ^{1}H-NMR; (δ, ppm) 8.38 (s, 1H, N=C-H), 7.4-7.6 (m, 4H, Ar), 7.2-7.5 (m, 4H, Ar), 7.0 (d, 2H, Ar), 6.4 (d, 2H, Ar), 4.8 (1H, OH), 4.1 (1H, NH). ^{13}C-NMR (δ, ppm); 163.8 (C=N), 161.2 (C=O), 160.6, 148.3, 141.6, 132.4, 128.6, 126.8, 125.2, 123.4, 121.2, 120.2, 118.6, 115.6, 115.2, 105.8.

3-Nitro-4-{4-[(3-nitro-benzylidene)-amino]-phenylamino}-chromen-2-one, 4c

Yield: 63.57 %, m.p.= 225-227 ºC, IR (KBr disc, cm^{-1}): 3455, 3081.54, 3072.38, 2952,84, 2364.72, 1687.18, 1629.75, 1615.48, 1552.32, 1347.86, 1205.48, 1065.88, 764,53. ^{1}H-NMR; (δ, ppm) 8.5 (s, 1H, N=C-H), 8.3-8.1 (m, 3H, Ar), 7.4-7.7 (m, 5H, Ar), 7.1 (d, 2H, Ar), 6.7 (d, 2H, Ar), 4.2 (1H, NH). ^{13}C-NMR (δ, ppm); 163.7 (C=N), 162.6 (C=O), 158.8, 148.4, 141.2, 134.6,131.6, 128.5, 127.4, 126.4, 125.2, 125.0, 124.6, 123.2, 121.8, 114.4, 103.2.

General procedure for preparation of 3-[4-(3-nitro-2-oxo-2H-chromen-4-ylamino)-phenyl]-4-phenylazetidin-2-ones, 5a-5c

In the solution that containing 0.5 mmol of the corresponding product **4a-4c** dissolved in 10 mL of benzene, 0.12 g (1.5 mmol) acetyl chloride was added. The mixture is stirred for 10 min at room temperature and then is refluxed for 12-13 hours. After cooling the product is concentrated and the remaining solid is dissolved in 5 mL of methanol, then heated to boiling and the excess of acetic acid is neutralized by adding 0.3 mmol sodium bicarbonate $NaHCO_3$ (controlled paper litmus until the solution undertake the blue color). The mixture is cooled in an ice bath and then filtered off under vacuum, then is washed with 2×1 mL of ether and dried in the air. The products are crystallized from methanol.

1-[4-(3-Nitro-2-oxo-2H-chromen-4-ylamino)-phenyl]-4-phenyl-azetidin-2-one, 5a

Yield: 45.85 %, m.p.= 236-238 ºC, IR (KBr disc, cm^{-1}): 3490-3400, 3241.24, 3086.34, 2933.88, 2379.36, 1687.18, 1611.68, 1588.55, 1516.35, 1422.67, 1328.68, 1206.66, 1062.47, 852.30, 756.92, 581.59. ^{1}H-NMR; (δ, ppm) 7.0-7.5 (m, 8H, Ar), 6.9 (d, 2H, Ar), 6.7 (d, 2H, Ar), 5.6 (t, 1H, N-C-H), 4.2 (1H, NH), 3.5 (d, 2H, CH_2). ^{13}C-NMR (δ, ppm); 172.5 (C=O), 162.6 (C=N), 161.8 (C=O), 149.4, 137.4, 130.2, 129.7, 128.5, 128.1, 127.3, 127.0, 126.7, 126.3, 125.8, 125.4, 124.6, 120.4, 114.7, 105.3, 55.4 (CH-N), 36.4 (CH_2).

4-(2-Hydroxy-phenyl)-1-[4-(3-nitro-2-oxo-2H-chromen-4-ylamino)-phenyl]-azetidin-2-one, 5b

Yield: 61.84 %, m.p.= 234-235 ºC, IR (KBr disc, cm^{-1}): 3540-3280, 3111.04, 2942.48, 2559.92, 2376.62, 1924.46, 1721.68, 1669.12, 1614.88, 1544.06, 1418.32, 1341.35, 1340.06, 1184.28, 1052.68, 1002.06, 886.64, 706.14, 654.26. ^{1}H-NMR; (δ, ppm) 7.1-7.4 (m, 7H, Ar), 6.8 (d, 2H, Ar), 6.6 (d, 2H, Ar), 5.7 (t, 1H, N-C-H), 5.1 (1H,OH), 4.0 (1H, NH), 3.6 (d, 2H, CH_2). ^{13}C-NMR (δ, ppm); 170.5 (C=O), 162.8 (C=N), 161.8 (C=O), 160.2, 156.4, 149.6, 130.2, 129.8, 129.3, 128.1, 127.4, 126.9, 126.5, 126.2, 125.7, 124.4, 124.1, 120.3, 120.0, 114.5, 114.2, 105.4, 56.5 (CH-N), 37.2 (CH_2).

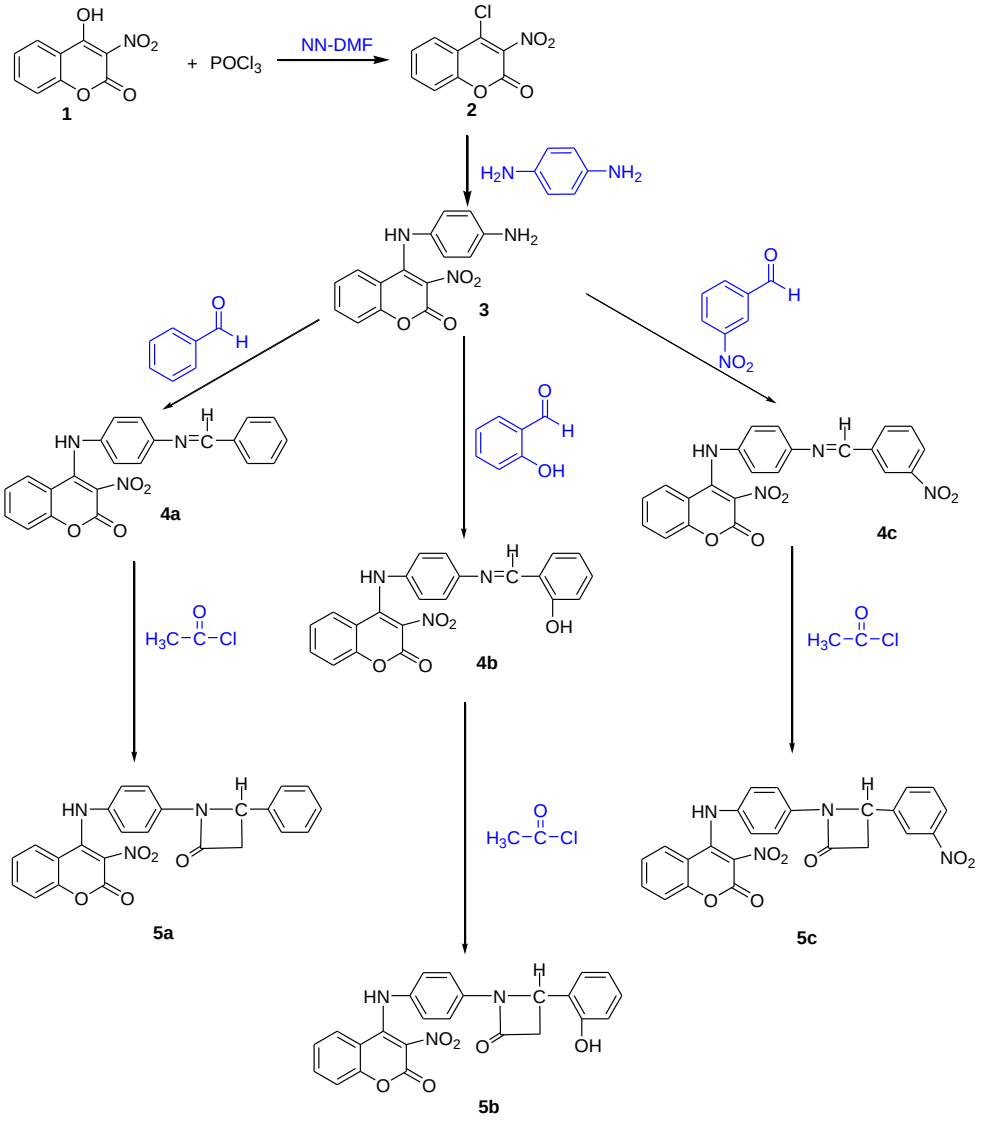
1-[4-(3-Nitro-2-oxo-2H-chromen-4-ylamino)-phenyl]-4-(3-nitro-phenyl)-azetidin-2-one, 5c

Yield: 39.68 %, m.p.= 228-229 ºC, IR (KBr disc, cm^{-1}): 3433, 3224.25, 3078.38, 2915.68, 2552.35, 2384.58, 1914.56, 1682.17, 1628.55, 1611.47, 1564.52, 1506.43, 1322.48, 1215.25, 1054.23, 836.07, 708.19, 581.16, ^{1}H-NMR; (δ, ppm) 7.1-7.7 (m, 7H, Ar), 6.7 (d, 2H, Ar), 6.5 (d, 2H, Ar), 5.8 (t, 1H, N-C-H), 3.9 (1H, NH), 3.7 (d, 2H, CH_2). ^{13}C-NMR (δ, ppm); 171.6 (C=O), 162.8 (C=N), 160.6 (C=O), 156.9, 152.4, 148.4, 137.4, 134.8, 131.6, 129.2, 128.6, 128.2, 127.1, 126.2, 125.3, 124.6, 123.8, 121.2, 120.8, 115.6, 105.4, 54.6 (CH-N), 38.6 (CH_2).

Results and discussion

During the condensation reactions of 4-chloro-3-nitrobenzopyran-2-one **2** and phenylenediamine, 4-(4-amino-phenylamino)-3-nitro-chromen-2-one, **3** is synthesized in good yield. By condensation reaction of product 3 and benzaldehyde, salicylaldehyde and 3-nitrobenzaldehyde, new derivatives of 4-[4-(benzylidene-amino)phenylamino]-3-nitrobenzopyran-2-ones, (**4a-4c**) are synthesized, as condensation products. In the last series of reactions, by cyclization of the product 4(a-c) and thioacetic acid, corresponding 1-[4-(3-nitro-2-oxo-2H-chromen-4-ylamino)phenyl]-azetidin-2-ones (**5a-5c**) are synthesized. Structural characterization of the synthesized products is based on spectrometric IR and NMR data. In the IR spectrum of the product, **3** appeared an absorption signal absorption at 3470.92 cm^{-1} which are responsible for ν(NH_2) stretching vibrations. The absorption signal at 3055.16 cm^{-1}, appeared due to ν(CH) stretching vibrations of the aromatic ring. The sharp peak at 1708.54 cm^{-1} region is responsible for ν(C=O) stretching vibrations, whereas the absorption peak at 1614.38 cm^{-1} region resulted from ν(C=C) stretching vibrations of the aromatic ring. The peak at 1511.25 cm^{-1} resulted from the absorptions of asymmetric ν(NO_2) stretching vibrations, while the peak at 1327.77 cm^{-1} resulted from symmetric ν(NO_2) stretching vibrations. On the other hand, the absorption peak at 1220.36 cm^{-1} is characteristic for vibrations of the lactonic stretching system (C-O-C), while the sharp peak at 750.71 cm^{-1} resulted from characteristic bending vibrations δ(C-H) oop of the aromatic ring.

In the IR spectrum of **4a**, an absorption signal appeared at 3461.45 cm^{-1} which are responsible for ν(NH) stretching vibrations, while absorption signal at 3075.24 cm^{-1} corresponds to ν(CH) of the aromatic ring. At 1694.15 cm^{-1} appeared the absorption signal which response to ν(C=O) stretching vibrations, whereas the sharp peak at 1642.76 cm^{-1} and a signal at 1603.07 cm^{-1} correspond due to ν(C=N) and ν(C=C) stretching vibrations of the aromatic system. The characteristic absorption at 1551.15 cm^{-1} resulted from asymmetric ν(NO_2) stretching vibrations whereas at 1326.42 cm^{-1} for symmetric ν(NO_2) stretching vibrations.



Scheme 1. Synthesis of azetidin-2-ones

Table 1. Physical properties of compounds **4a-4c** and **5a-5c** and their elemental analysis

Nr.	Formula	M_{wt}	Elemental analysis (%),calc / found	M.p., ºC	Yield, %
4a	$C_{22}H_{15}N_3O_4$	385.37	(C-68.55; H-3.93; N-10.90; O-16.62) (C-67.89; H-3.75; N-10.46)	224-226	87.26
4b	$C_{22}H_{15}N_3O_5$	401.37	(C-68.82; H-3.77; N-10.47; O-19.94) (C-68.03; H-3.34; N-10.18)	205-207	39.88
4c	$C_{22}H_{14}N_4O_6$	430.37	(C-61.38; H-3.28; N-13.02; O-22.31) (C-61.02; H-3.12; N-12.89)	225-227	63.57
5a	$C_{24}H_{17}N_3O_5$	427.41	(C-67.44; H-4.01; N-9.83; O-18.72) (C-66.98; H-3.96; N-9.78)	236-238	45.85
5b	$C_{24}H_{17}N_3O_6$	443.41	(C-65.01; H-3.86; N-9.48; O-21.65) (C-64.78; H-3.91; N-9.39)	234-235	61.84
5c	$C_{24}H_{16}N_4O_7$	472.41	(C-61.01; H-3.41; N-11.86; O-23.71) (C-60.59; H-3.32; N-11.34)	228-229	39.68

Absorption at 1221.34 cm^{-1} is also characteristic for stretching(C-O-C) vibrations of the lactonic ring, whereas the sharp peak at 756.85 cm^{-1} is characteristic for bending δ(C-H) oop vibrations of the aromatic ring. On the other hand, signals from ^{1}H-NMR spectrum correspond to the absorption of respective protons. A proton singlet displayed at 8.35 ppm resulting from N=C-H. Also in the ^{13}C-NMR spectrum is displayed a signal at 165.4 ppm which corresponds to the C=N carbon.

The IR spectra of compound **4b**, showed the absorption signal at 3451.94 cm^{-1} which is responsible for ν(NH) stretching vibrations, the broad band at 3307.35 cm^{-1} correspond to stretching ν(OH) absorption, and a signal at 3079.24 cm^{-1} resulted due to ν(CH) stretching vibrations of the aromatic ring. The sharp peak at 1684.15 cm^{-1} resulted from ν(C=O) stretching vibrations, whereas signals at 1608.97 and 1552.51 cm^{-1} result from ν(C=N) and ν(C=C) stretching vibrations. The peak at 1514.34 cm^{-1} corresponds to absorptions of asymmetric ν(NO_2) stretching vibrations, while the one at 1320.49 cm^{-1} due to symetric ν(NO_2) stretching vibrations. Absorption signal at 1211.64 cm^{-1} is characteristic for stretching vibrations of the lactonic (C-O-C) system, and the sharp peak at 761.67 cm^{-1} resulted from δ(C-H) bending oop vibrations of the aromatic ring. In the ^{1}H-NMR spectrum, besides multiplets of aromatic protons, a proton singlet resulting from N=C-H appears at 8.38 ppm. In the ^{13}C-NMR spectrum are also displayed a signal at 163.8 ppm, which correspond to C=N carbon.

The IR spectra of **4c** showed an absorption peak at 3455 cm^{-1} which are responsible for ν(NH) stretching vibrations, while the absorption signal at 3081.54 cm^{-1} resulted from ν(CH) aromatic vibrations. The peak at 1687.18 cm^{-1} is responsible for absorbing the ν(C=O) stretching vibrations whereas two signals at 1629.75 and 1615.48 cm^{-1} results from ν(C=N) and ν(C=C) stretching vibrations of the aromatic ring. The sharp peak in the wavelength of 1552.32 cm^{-1} resulted due to asymmetric ν(NO_2) stretching vibrations, while absorption signal at 1347.86 cm^{-1} reflects symmetric ν(NO_2) stretching vibrations. A signal at 1204.76 cm^{-1} is characteristic for (C-O-C) stretching vibrations of the lactonic system, while the sharp peak at 764.53 cm^{-1} appeared from δ(C-H) bending oop vibrations of the aromatic ring. In the ^{1}H-NMR spectrum, the multiplet signals of aromatic protons appeared at 8.1-8.3 ppm and 7.7-7.4 ppm. A proton singlet resulting from N=C-H appears at 8.5 ppm. In the ^{13}C-NMR spectrum also displayed a signal at 163.7 ppm, that correspond to C=N and a signal at 162.6 ppm resulted from C=O carbon.

In the IR spectra of the compound **5a**, a sharp absorption signal appeared at 3490 cm^{-1}, which is responsible for ν(NH) stretching vibrations whereas the absorption peak at 3086.34 cm^{-1} resulted from ν(CH) vibrations of the aromatic system. The medium band at 2933.88 cm^{-1} resulted from the absorptions of ν(CH) stretching vibrations of aliphatic protons, whereas the sharp peak at 1687.18 cm^{-1} from ν(C=O) stretching vibrations. The absorption signal at 1611.68 cm^{-1} resulted from ν(C=C) stretching aromatic vibrations. The characteristic signals of the nitro group appeared at 1516.35 cm^{-1} due to asymmetric stretching, whereas at 1328.68 cm^{-1} for symmetric ν(NO_2) stretching vibrations. The absorption peak at 1206.66 cm^{-1} resulted from lactonic ν(C-O-C) stretching vibrations, whereas at 756.92 cm^{-1} appeared the absorption signal resulted from δ(CH) oop vibrations of the aromatic system. In the ^{1}H-NMR spectra, a proton singlet at 5.6 ppm resulted from N-C-H, while two doublets at 6.7 and 6.9 ppm and a multiplet at 7.5-7.0 ppm correspond to aromatic protons. A doublet at 3.5 ppm resulted from aliphatic CH_2 protons of the azetidinone system. ^{13}C-NMR spectra appeared three peaks at 172.5, 162.6 and 161.8 which results from C=O and C-N carbons, whereas a signal at 55.4 ppm resulted from the CH-N of the azetididinone ring. An absorption signal at 36.4 ppm appeared due to methylene carbon.

In the IR spectra of the compound **5b,** a broad absorption signal appeared at 3540-3280 cm^{-1} which are responsible for ν(OH) stretching vibrations and the absorption signal at 3111.04 cm^{-1} for ν(CH) stretching vibrations of the aromatic ring. The peak at 2942.48 cm^{-1} resulted from the absorptions ν(CH) stretching vibrations of the methylene group, while at the peak of 1721.68 cm^{-1} correspond to ν(C=O) stretching

vibrations. The characteristic peak at 1669.12 cm^{-1} resulted from ν(C=C) stretching vibrations of aromatic moiety. Signals at 1544.06 and 1341.35 cm^{-1} appeared due to ν(NO_2) asymmetric and symmetric stretching vibrations, whereas the characteristic signal at 1184.28 cm^{-1} is responsible for lactonic ν(C-O-C) vibrations. In the ^{1}H-NMR spectra are shown characteristic signals at 3.6 ppm (d, 2H, CH_2), and at 5.7 ppm (t, 1H, N-C-H). ^{13}C-NMR spectra also showed a signal at 162.8 (C=N) and absorptions at 56.5 ppm (CH-N) and 37.2 ppm (CH_2).

Table 2. Zones of inhibition (mm) of the discs impregnated with various concentration of the synthesized compounds

No.	*S. aureus*			*E. coli*			*Klebsiella*		
	2 mg mL^{-1}	4 mg mL^{-1}	6 mg mL^{-1}	2 mg mL^{-1}	4 mg mL^{-1}	6 mg mL^{-1}	2 mg mL^{-1}	4 mg mL^{-1}	6 mg mL^{-1}
4a	6.5	7.0	8.0	6.0	6.5	8.0	6.0	6.0	6.5
4b	6.5	8.0	8.5	7.5	8.0	6.5	7.5	7.5	8.5
4c	7.0	7.5	8.5	7.0	8.0	9.0	6.5	7.0	9.0
5a	8.0	8.5	9.5	9.0	9.5	11.0	10.0	10.5	13.0
5b	11.5	13.5	15.5	9.0	10.0	12.0	9.0	10.0	12.0
5c	9.0	12.0	14.0	9.5	8.5	9.5	7.5	8.0	9.0

Compound **5c** appeared the absorption signal at 3433 cm^{-1} which is responsible for ν(NH) stretching vibrations and the absorption signal at 3078.38 cm^{-1}, which resulted from aromatic ν(CH) stretching vibrations. The peak at 2915.68 cm^{-1} resulted from ν(CH) stretching vibrations of the methylene group, while the signal at 1682.17 cm^{-1} absorption reflects the vibrations ν(C=O) stretching. The peak at the wavelength of 1628.55 cm^{-1} resulted from vibrations ν(C=C) stretching mode. The absorption peak at 1506.43 cm-1 resulted from ν(NO_2) asymmetric stretching vibrations, while symmetric ν(NO_2) stretching vibrations resulted by absorption peak at 1322.48 cm^{-1}. The absorption signal at 1215.25 cm^{-1} is characteristic for (C-O-C) stretching vibrations of the lactonic system, while the sharp peak at 708.19 cm^{-1} is characteristic for δ(C-H) oop bending vibrations of the aromatic ring. On the other hand, characteristic signals from the ^{1}H-NMR spectrum, a triplet at 5.8 ppm and a doublet at 3.7 pm correspond to proton absorptions of N-C-H and CH_2 of the azetidinone ring. Also in the ^{13}C-NMR spectra, the azetidinone carbon signals appear at 54.6 ppm (CH-N) and 38.6 ppm, (CH_2).

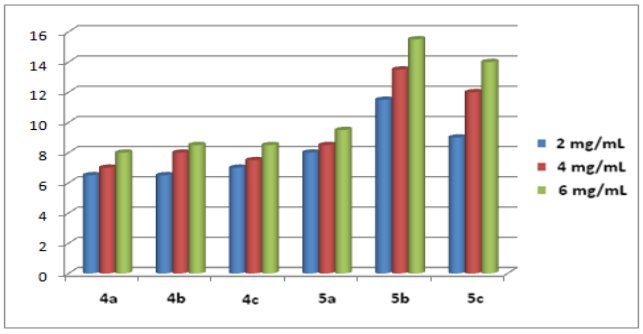


Figure 1. The graphical presentation of zone of inhibition (mm) against *S. Aureus.*

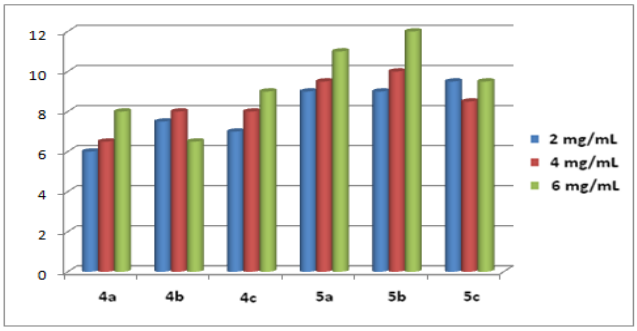


Figure 2. The graphical presentation of zone of inhibition (mm) against *E. Coli.*

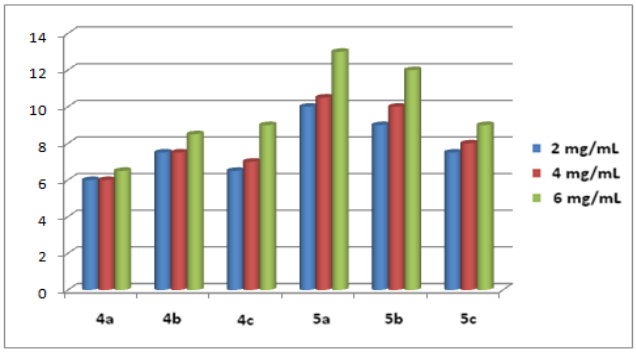


Figure 3. The graphical presentation of zone of inhibition (mm) against *Klebsiella.*

Antibacterial activity of the products 4a-4c and 5a-5c

Following this study, compounds **4a-4c** and **5a-5c** are investigated for their antibacterial activity. Our research is oriented to test the activity against bacteria *S. aureus*, *E. coli*, and *Klebsiella*, on the basis of Standard Disc Method,[24] by measuring the zones of inhibition around the standard discs. The discs have previously been impregnated with solutions of the products in DMF with concentrations of 2 mg mL^{-1}, 4 mg $mL^{-1}$and 6 mg mL^{-1}. Results are shown in Table 2.

Compounds of series **4** showed moderate antimicrobial activity against these microorganisms, whereas those of series **5** exhibited significant activity. Products **5b** and **5c** were most active against *S. aureus*, compounds **5b** and **5a** showed the most activity against *E. Coli* whereas **5a** and **5**b were more active against *Klebsiella*.

Antibacterial activity against *E. Coli* and *Klebsiella* appeared as bactericide activity is displayed in large-scale. Furthermore, these compounds expressed both bacteriostatic and bactericide activity against *S. Aureus*.

Bacteriostatic activity is exhibited in a large range (+2.0 mm), whereas bactericide activity showed in small diameter. Azetidin-2-one moiety showed significant impact on antimicrobial activity, whereas the impact of polar groups also was distinct. It is particularly noted the impact of the hydroxy group of **5b**, which has affected the increase in antibacterial activity. Moreover, nitro group of **5b** has shown significant impact on the range of inhibition of *E. coli*.

The assumption is that antibacterial activity may result as a consequence of the involvement of these products in enzymatic reactions. These products can cause enzymatic inhibition, by inhibiting cell wall construction of the microorganisms. However, mechanism of enzymatic inhibition is not yet fully studied. In general, by increasing the concentration of solvents, their antimicrobial activity increased.

Conclusions

New derivatives of 4-[4-(benzylidene-amino)-phenylamino]-3-nitrobenzopyran-2-ones, **4a-4c** and respective azetidin-2-ones, **5a-5c** are synthesized in the moderate and high yield. Compounds **5b** and **5c** were most active against *S. aureus*, compounds **5b** and **5a** showed more activity against *E. Coli*, while **5a** and **5b** were more active against *Klebsiella* bacteria. The impact of polar groups in antibacterial activity was significant. Antibacterial activity is shown to be proportional to the concentration of these compounds.

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ECOLOGICAL AND ECONOMIC ASPECTS OF ELECTROCHEMICAL PURIFICATION OF QUARRY WATERS FROM THE IONS OF HEAVY METALS

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Keywords:electrochemical processing; quarry waters; copper

The problems of purification of quarry waters from the ions of heavy metals by the use of electrochemical method are considered and a construction of electrochemical reactor is described allowing to perform the so-called channel electrolysis at a high rate of liquid flow along of the electrodes in the conditions of limiting cathode current density (J_k). Technological schemes of complex processing of quarry waters for separation of useful components and estimation of their economic efficiency are presented.

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INTRODUCTION

The problem of extraction of heavy metals from the sewage of mining production is very daunting in industrial regions.[1] In the process of long-term storage of the residues of processing of polymetallic sulfide ores, under the action of air oxygen, the gradual oxidation of sparingly soluble sulfides of heavy metals takes place with a formation of readily soluble compounds - sulfites and sulfates. These oxidation products penetrate to undersoil mine and shaft waters as a result of leaching with a consequent environmental contamination.

The ions of heavy metals, by a stress indicator, by their harmful environmental effect are even ahead of the residues of nuclear power plants and entities producing the organic compounds. Biosphere contamination by the ions of heavy metals is at the first place and is estimated as 135 points in the scale of ecological stress-factor, whereas an environmental contamination by radiation residues is estimated as 40 points by the same scale.[2] Therefore purification of the quarry waters (QW) from the ions of heavy metals is primarily considered as the ecological problem. From another hand, QW which contain a significant amount of valuable components, and in connection with their high cost, may be considered as a raw material for the production of a marketable product. Elaboration of profitable technology for their extraction from mentioned waters by substantial economic effect is a highly actual problem.

The problem of purification of QW from the ions of heavy metals is also actual for Bolnisi Region of Georgia. In this region QW contain toxic ions, an amount of which is significantly higher than limiting permissible concentration (LPC):

Table 1. Content of metal ions in QW of Bolnisi Region.

Element	Cu	Zn	Fe	Cd	pH
kg ·m^{-3}	0.8 ÷1.2	0.4 ÷0.7	1.0 ÷1.4	0.04	2.5

Annually from quarry streams the following is removed (in tons): Cu - 1290; Zn - 430÷760; Fe - 1100÷1500; Cd - 4.34; SO_4^{2-} ions - 4400. Therefore QW, containing the significant amount of useful components should be considered as the anthropogenic raw material, and at their complex processing, the considerable profit may be obtained besides the solving of a major ecological problem.[3.]

The copper ions are one of the primary sources of hydrosphere contamination by the ions of heavy metals. Purification of QW from copper ions in Join-Stock Company “Madneuli” (Georgia) is mainly carried out by cementation method which is slightly improved in technological viewpoint by the fact that a low-grade iron powder is used instead of steel chips and scrap. As a result, for deposition of 1 ton of copper powder more than 2 tons of iron precipitator is used, whereas the theoretical consumption comprises 0.9 tons. Obtained product involves a low-grade concentrate containing 50-60 % of copper and 15-20 % of iron. Separation of pure copper from mentioned concentrate is a very complicated problem and obtaining of any other product is practically impossible. The greater is an oxidized iron and large granules in the powder the more significant amount of iron passes to the solution by chemical dissolving, and the smaller amount of iron participates in copper precipitation. This process takes place in the practice of Join-stock Company “Madneuli". Moreover, the technology is oriented to water purification only from copper ions.

Direct electrolysis (cathodic precipitation) is among the most promising methods of purification of the quarry and industrial sewage from copper ions, allowing the metal extraction from aqueous solutions as a goal product. In this case, at the cathode, the pure copper powder is separated (it's processing to useful products isn't a complex problem) and at the anode a neutralization of toxic components,

existing in the solution, takes place. Electrolysis performance from concentrated solutions is not a particular challenge. Obtaining of electrolytic copper powder from diluted solutions, containing [Cu] ≤1 $g \cdot L^{-1}$, involves some difficulties.

Therefore electrolysis method doesn't receive a widespread use for copper extraction from diluted solutions (because of low current efficiency and polarization and, hence, because of high voltage at electrolyzer). But the problem is entirely solvable at the creation of corresponding construction of electrochemical reactor which will allow the performance of so-called channel electrolysis, that is to say, an electrolysis at a high rate of fluid motion along the electrodes and in the conditions of limiting current density (J_k).

Over the years, our Institute has been working on the elaboration of such electrolyzer but the mentioned plants had certain disadvantages.[4,5]

EXPERIMENTAL

For copper extraction from diluted solutions ([Cu] ≤ 1 $g \cdot L^{-1}$) by high degree and high current efficiency we have elaborated the original construction of basic electrolyzer with a corresponding hydrodynamic regime (Fig. 1).[6,7] Electrochemical reactor involves the cylindrical body with a conic bottom. In reactor body, a cassette with a radial located fixed electrodes of definite shape is inserted. Manganese titanium dioxide is used as anode material, characterized by high durability in the conditions of long-term operation of an electrolyzer.

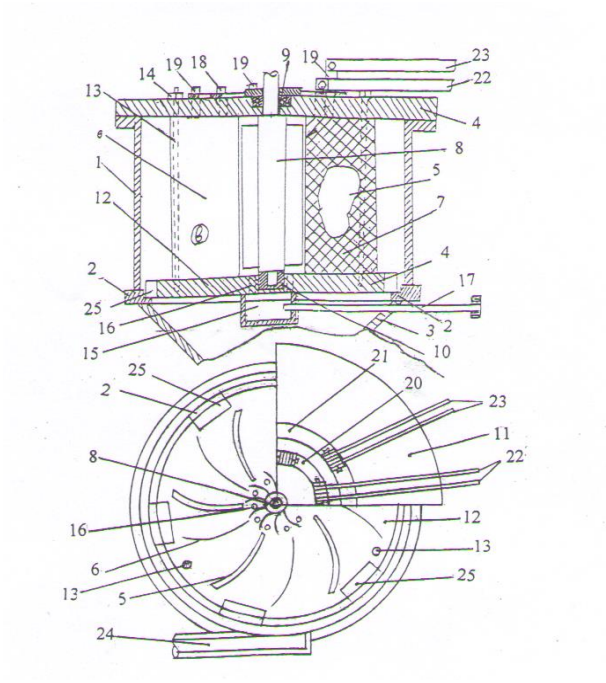

Figure 1. The electrochemical reactor. 1. body; 2. shelf; 3. conic bottom; 4. magazine; 5. anodes; 6. cathodes; 7. slots; 8. mixer; 9,10. bearings; 11,12. upper and lower plates; 13,14. bolts and nuts; 15. box; 16. holes; 17. pipe; 18,19. current lead; 20,21. tires current lead; 22,23. tires rectifier; 24. trough; 25. window.

The anodes are enclosed in an acid-resistant bag to avoid the permanent recharge iron ions existing in water. As a result, the power consumpion reduces by 5-15 %. The plates from stainless steel were used as the cathodes. In cassette center, the mixer is located which directs a liquid flow between the electrodes by a high rate. The electrode shape is favorable for the increase of the distance between opposite electrodes from the center to the periphery, on one side.

This fact allows the reducing of current density on the electrodes to the same direction. Simultaneously the depolarizer concentration is decreased from the center to the periphery. Thus an automatic control of the correspondence between concentration and current density takes place which is favorable for maintenance of product maximum current efficiency.

Extraction of electropositive metals is performed in the regime of limiting current. As a result, the spongy precipitate is formed. On the other hand, electrode shape is favorable for the conservation of liquid flow of circular motion. The reactor operates by the hydro-cyclone principle and liquid flow, moving along the electrodes by high rate, removes the cathode product and involves it to a circular motion on the inner surface of cylindrical reactor body. The particles of copper powder lose a rate and penetrate into the collector through the holes on reactor bottom. In the reactor, a significant improvement of the intensity of forced convection and solving the problem of removal of copper powder from the cathodes is attained.

The technological process is continuous, and a reactor is similar to an (electric) filter, in which its selective extraction takes place at the passing of the solution, contaminated by toxic copper ions. Precipitate dispersity increases the area of its surface significantly and decreases the real current density considerably on the electrode surface. The voltage on reactor clamps is no more than 2-3 V. Obtained product - copper contains only the oxygen as an impurity which is easily removed at product treatment in reducing medium.

RESULTS AND DISCUSSION

Cascade arrangement of two mentioned electrochemical reactors, one of which operates at high current densities and another at relatively low ones, gives 95 % copper extraction from QW (Table 2). Power consumption, in this case, comprises 4000 $kW \cdot hour \cdot ton^{-1}$, corresponding to 5-10 % of the copper cost. At remelting of electrolytic copper powder, a copper ingot of 99.0 % was obtained

For complex processing of QW for the purpose of extraction of useful components and their further processing for obtaining of valuable, marketable products, we have elaborated several technological schemes (Fig. 2).

Considering QW as the technogenic raw material the following marketable - commercial products may be obtained by mentioned schemes: copper ingots of 99.8 %; vitriol and other chemical compounds; bio dye and other pigments; zinc and its powder; gold; pure secondary water.

Table 2. Experimental results at cascade arrangement of two reactors.

No.	QW feed, $L \cdot h^{-1}$	Current,A	Voltage, V	Time, h	Cu, $g \cdot L^{-1}$		Cu removal, %	Current efficiency, %	Specific power consumption, kWh^{-1}
					initial	final			
1	12	15	2.8	3.5	1.08	0.159	85.2	62.1	3800
2	12	4	2.4	3.5	0.159	0.05	68.6	27.5	7339
				Total			95.4	54.9	4175

Table 3. Estimation of economic efficiency (1000 USA dollars) of technological schemes of processing of QW.

	I	Pr	PA	MC	Product price		Gain from product		Additional cost		Taxes		Annual profit	
					O	P	O	P	O	P	O	P	O	P
# 1	800	Cu (99.8%)	830	600	6111	5482	5072	4550	4472	3950	1582	1412	2890	2538
# 2	700	Vitriol (99.6%)	3300	700	2413	2300	9344	8796	8644	8096	2977	2797	5667	5299
		Gold	1065*		1222	1133								
# 3	1100	Copper	830	1000	6111	5482	6266	5584	5260	94584	1916	1693	4344	2891
		Zinc	400		2971	2587								
# 4	1000	Vitriol	3300	1100	2413	2300	10532	9757	8900	8657	3231	3055	5670	5602
		Gold	1065*		1222	1133								
		Zinc	400		2971	2587								

O-optimistic estimation; P-pessimistic estimation; *in ounce, #1and #2; short and #3and #4 are full technologies. The prices for corresponding metals are taken from official data of London Exchange on 14.02.2017, on other products – by corresponding tariffs. I-investment, Pr-product, PA product amount in ton, MC- maintenance cost

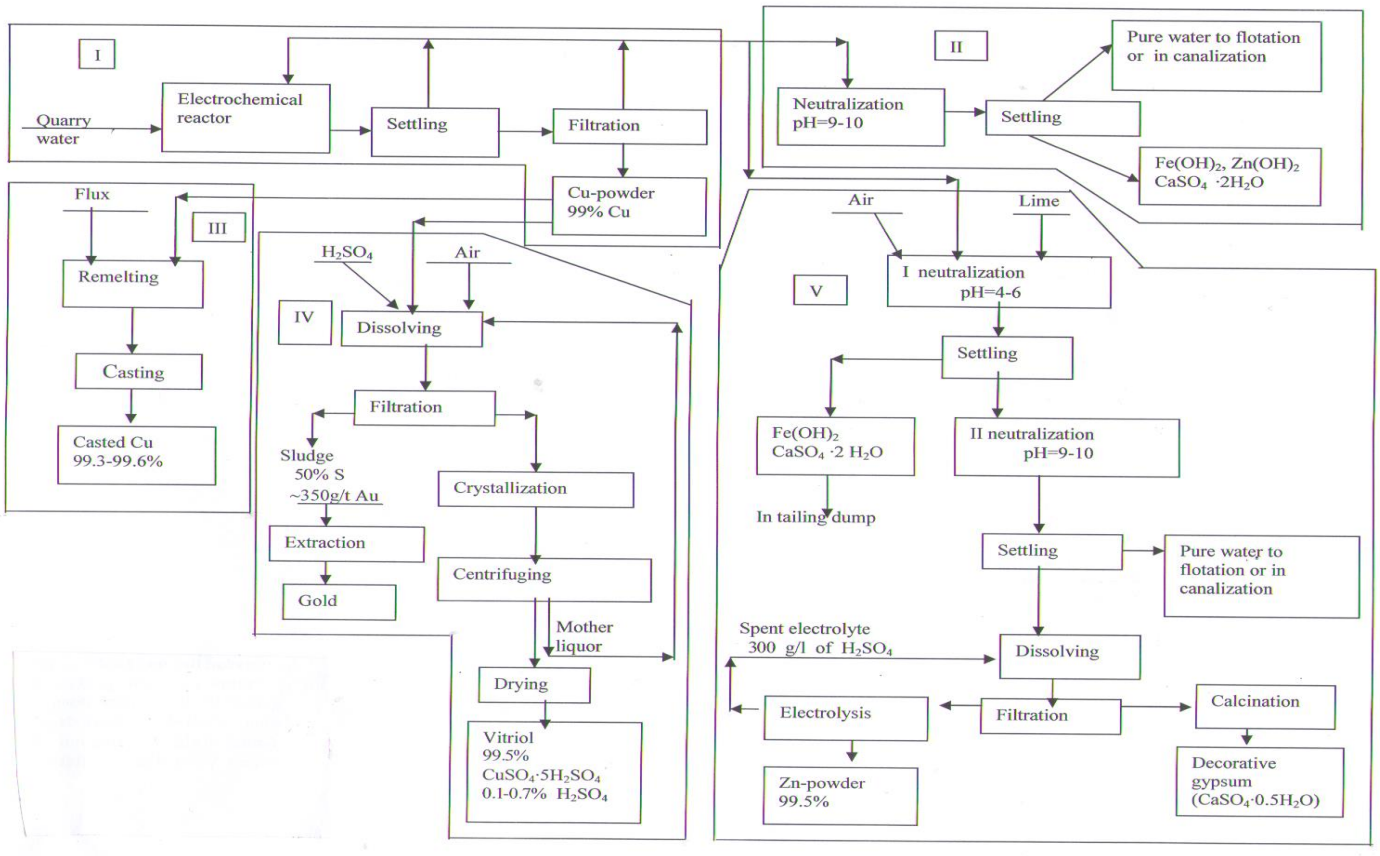

Figure 2. Short and full Schemes of processing of quarry water. 1. short scheme _ I+II+III (units); 2. short scheme _ I+II+IV(units); 3. full scheme _ I+III+V(units); 4. full scheme _ I+V+IV(units).

The majority of these products are scarce. Therefore their realization is beyond question. When it is considered that the main raw material – QW is free of charge and field cost are relatively small, the price cost of obtained production is low. This fact increases its competitive ability significantly.

The scheme of processing of electrolytic copper is best suited for obtaining of vitriol – traditional fungicide for Georgian agriculture. Vitriol preparation was performed by conventional, but significantly simplified technology. Obtained vitriol contains a negligible impurity and is characterized by dendrite structure with developed surface.

In Table 3 the estimation of economic efficiency of QW processing by proposed schemes is presented. In the case of full-scale realization of proposed technology annually obtaining of the profit 8-9 million US $ is possible Table 3.

CONCLUSIONS

1. Construction of electrochemical reactor has been elaborated allowing to perform the process of purification of quarry waters from copper ions by high efficiency (copper extraction by 95 %) at power consumption corresponding to 5±10 %.

2. Technological schemes were elaborated for complex processing of quarry waters for separation of useful components for their processing into costly marketable production.

3. It was shown that at a full-scale realization of elaborated technological schemes an annual profit of 8-9 million US dollars might be obtained.

4. The hydrometallurgical technology of sewage purification of our elaboration belongs to so-called self-financing technologies. Even insignificant investment initiates a self-financing.

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