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Antiinflammatory activity of aqua(cresoxyacetato)-copper(II) complexes

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It is known that low molecular weight (carboxylato)-copper(II) complexes can be beneficial in influencing inflammation reactions [1–4]. The antiphlogistic activities of (phenoxyacetato)-, (chlorophenoxyacetato)- and (naphthoxyacetato)copper(II) aquacomplexes were assayed in rat paw dextran- or carrageenan-indued edemas [5, 6]. This paper is devoted to the study of antiedematous activity of the mononuclear aquabis(cresoxyacetato)copper(II) complexes with $[Cu(H_2O)_n(ROCH_2COO)_2]$ composition, where R = 2-methylphenyl (n = 2, complex **1**); 3-methylphenyl (n = 2, complex **2**) and 4-methylphenyl (n = 3, complex **3**), including the corresponding isomeric cresoxyacetic acids (**1a**–**3a**).

On the basis of the different coordination of $ROCH_2COO^-$ acidoligands the complexes **1**–**3** belong to two groups of mononuclear (aryloxyacetato)copper(II) complexes with tetragonal symmetry [7]. In diaquacomplexes **1** and **2** the acidoligands are coordinated by a chelate mode to Cu(II) atom via both carboxylate and ether oxygen atoms yielding the similar molecular structure as it was found for diaquabis(phenoxyacetato)copper(II) [8]. On the other hand, triaquacomplex **3** is represented by a square-pyramidal structure with the monodentate acidoligand coordination which is typical for phenoxyacetate copper(II) trihydrate [9].

Using a routine plethysmometric method, the evaluation of antiedematous activity of all compounds was carried out in the rat paw carrageenan-induced edema model (Table). The effects of the tested Cu(II) complexes **1**–**3** were compared to those of the free isomeric cresoxyacetic acids **1a**–**3a**. Aqua(cresoxyacetato)copper(II) complexes are clearly more effective than the acids, with the exception of a pair of the ortho derivative. The average antiinflammatory activities of the compounds (ordered by a measure of the effect of complexes) decreased in the following order: **2/2a** (71.0/37.1%) ≈ **3/3a** (70.8/46.5%) > **1/1a** (43.0/46.5%). In the case of complex **1**, the increased stability of $[Cu(H_2O)_n(ROCH_2COO)_2]$ species under *in vivo* conditions could be explained by the ortho-effect of the methyl group protecting the chelate coordination of $ROCH_2COO^-$ acidoligands in ether moiety against the aquation reactions. In contrast, complexes **2** and **3** are bioavailable to the formation of pharmaco-active forms though the controlled liberation of aryloxyacetate and Cu^{2+} ions. These two complexes were more active than a salicylate pair – salicylic acid (mean edema reduction 40.7%) and dihydrate diaquabis(salicylate)copper(II) complex (57.4%) – under the same conditions [6].

Table: Antiinflammatory activity of compounds 1–3 and 1a–3a

Compd.	Edema volume changes ΔV (±SEM) (cm^3)						
	Time interval (min)						
	30	60	120	180	240	300	360
CG	0.14 (0.03)	0.24 (0.03)	0.34 (0.04)	0.32 (0.02)	0.33 (0.03)	0.35 (0.04)	0.38 (0.04)
1	0.07 (0.01)	0.18 (0.02)	0.25 (0.02)	0.21** (0.02)	0.20* (0.04)	0.15** (0.04)	0.12** (0.05)
1a	0.15 (0.03)	0.30 (0.03)	0.15** (0.02)	0.13** (0.01)	0.09** (0.02)	0.05** (0.02)	0.06** (0.02)
2	0.09 (0.01)	0.14 (0.02)	0.12** (0.02)	0.07** (0.01)	0.04** (0.01)	0.02** (0.01)	0.02** (0.01)
2a	0.11 (0.02)	0.20 (0.04)	0.32 (0.03)	0.25 (0.04)	0.17** (0.04)	0.11** (0.03)	0.10** (0.03)
3	0.09 (0.00)	0.15 (0.00)	0.11** (0.01)	0.07** (0.01)	0.04** (0.01)	0.20** (0.01)	0.20** (0.01)
3a	0.07 (0.02)	0.13* (0.01)	0.15** (0.01)	0.18 (0.02)	0.23 (0.03)	0.21* (0.04)	0.20* (0.04)

CG control group of animals (n = 11); statistical significance * $P < 0.05$, ** $P < 0.02$ (n = 8)

Experimental

The complexes **1**–**3** and the corresponding carboxylic acids **1a**–**3a** were used for biological tests. Their preparation and basic physico-chemical characterization were published previously [6]. All compounds were dispersed in sterilized saline with a concentration of 50 μmol/cm^3 (calculated for aryloxyacetate fragment) and stabilized by 0.05% Tween 80 (Merck). Wistar male and female rats (Velaz, Prague), weighing 230 ± 20 g, were used. Acute antiedematous activity (Table) was measured by reduction of rat paw edema, induced by injection of 0.1 ml of 1% carrageenan (Serva) in sterilized saline. The tested compounds were applied i.p. in a single dose of 50 μmol/kg body weight, 30 min before injecting the irritant substance [6]. Control animals received only vehicle. The changes of edema volume were evaluated plethysmometrically [10]. Statistical significance of results was established using the Student's t-test. All differences were considered significant at $P < 0.05$.

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Antifungal activity of 2′-substituted furanocoumarins and related compounds

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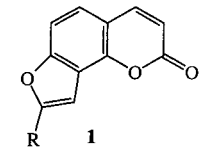
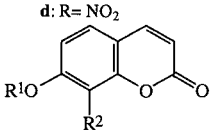
With the advent of the AIDS era in the 1980's, a broad range of fungal infections are being reported in the medical practice. *Candida* species are now ranked as the third most common causative agent of nosocomial blood stream



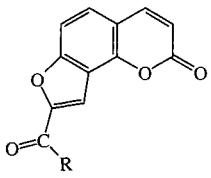
1

a: R = H
b: R = COOH
c: R = COOEt
d: R= NO_2



2

b: R^1 = H, R^2 = H
c: R^1 = H, R^2 = I
d: R^1 = $CF_3C_6H_4CO$, R^2 = H



8, 9

8a: R = N LeuOEt
8b: R = N Leu
8c: R = N PheOEt
8d: R = N Phe
8e: R = N Z-LysOMe
8f: R = N Z-Lys
9a: R = $NH(CH_2)_2CH_3$
9b: R = $NH(CH_2)_9CH_3$
9c: R = $NH(CH_2)_{15}CH_3$
9d: R = $NH(CH_2)_{10}NH_2$
9e: R = $NH(CH_2)_{10}COOH$
9f: R = $NH(CH_2)_{10}COOEt$
9g: R = $NC_6H_{11}CONHC_6H_{11}$

infections in most hospitals [1, 2]. Emergence of new resistant species of fungi in addition to the poor safety profile of present antifungal drugs are the main reasons for discovery of new prototype molecules.

Coumarins are a group of induced antifungal compounds in plants [3]. Coumarin inhibits the germination of spores of *Aspergillus niger, Penicillium glaucum,* and *Rhizopus nigricans* [4]. Novobiocin and other 4-hydroxycoumarins are generally ineffective against fungi [5]. According to our previous study, angelicin (**1a**) was isolated as the antifungal constituent of *Diplotaenia damavandica* a rare Iranian native plant [6]. To improve the potency and antifungal profile of this lead structure, different modifications were considered based on the strategy of "mimicry mixed functionality".

The antifungal activity of different coumarin derivatives is shown in the Table. The activity of simple segments, i.e., coumarin (**2a**) and benzofuran (**3**) are much less compared with the original furanocoumarin, angelicin. Other compounds with phenolic hydroxyl group, esculetin (**2e**), 3,4-dihydroxybenzoic acid (**4a**), gallic acid (**4b**), pyrogallol (**4c**), and umbelliferone (**2b**), do not show promising activity either. In this respect, pyrogallol has the strongest effect. In a study by Dini et al. [7] it was shown that an aromatic hydroxyl group and/or an extra oxygenated functional group (ether or ester) in 6 and 7 positions of coumarin were necessary for the antifungal activity. Alkylated derivatives of 7-hydroxycoumarin may show both antifungal and antibacterial properties. A free hydroxyl group at position 7 of coumarin nucleus is important for antibacterial activity. It has been suggested that the antifungal activity of furanocoumarins may be accounted for by protection of the phenolic OH at position 7 by the etheric bond [5].

The 7-substituted coumarins, **2b**–**2d**, are either very weak or inactive on *C. albicans*. Esterification of **2b** by a pharmacophoric group such as $CF_3C_6H_4CO$ to produce **2d** does not improve the activity. The carbonyl group was used to link different moieties to the furanocoumarins nucleus. Although the carboxyl derivative **1b** is not considered active, the ethyl ester derivative **1c** gained some activity. Compound **1d** is a potent antifungal and by having its low synthetic yield improved, this compound could be a precursor for more derivatives.

Since we have already reported the antifungal activity of simple long chain acids [8], it was decided to determine the amine-containing analogs and their effect when attached to a coumarin nucleus. Among the alkylamines, propylamine (**5a**) decylamine (**5b**) and hexadecylamine

Table: *In vitro* antifungal activity of the coumarins and other compounds, expressed as MIC values (µg/ml)

Compd.	1[a]	2[b]	3[c]	4[d]
1a	62.5	250	125	2.5
1b	>2000	2000	–	2000
1c	1000	250	–	1000
1d	15.6	3.9	<1.9	15.6
2a	>1000	500	1000	–[e]
2b	1000	500	–	500
2c	1000	500	–	1000
2d	>500	500	500	–
2e	>1000	1000	1000	–
3	1000	500	500	–
4a	>1000	>1000	>1000	–
4b	500	250	1000	–
4c	250	125	–	–
5a	125	62.5	250	>500
5b	<7.8	<7.8	<7.8	15.6
5c	15.6	<1.9	<1.9	7.8
6a	500	250	500	500
6b	62.5	15.6	7.8	62.5
6c	500	125	250	250
7	250	125	125	125
8a	>1000	1000	–	>1000
8b	>2000	1000	–	2000
8c	>2000	2000	125	1000
8d	>500	500	250	125
8e	>1000	>1000	>1000	1000
8f	>500	>500	500	125
9a	250	125	62.5	>250
9b	250	31.3	62.5	125
9c	>250	31.3	125	250
9d	250	250	500	500
9e	500	>500	250	>500
9f	>250	125	>250	>250
9g	>500	>500	>500	>500
Fluconazole	12.8	25.6	6.4	10.0

[a] *Candida albicans* ATCC 14053; [b] *Cryptococcus neoformans* KF-33; [c] *Saccharomyces cerevisiae* PLM 454; [d] *Aspergillus niger* PLM 1140; [e] "–", not tested

(**5c**) and other long chain derivatives, 11-aminoundecanoic acid (**6a**) 11-aminoundecanoic acid ethyl ester (**6b**), 1,10-diaminodecane (**6c**), and spermin (**7**), there are several active molecules. In the alkylamine series, **5c** possesses a very strong antifungal activity, especially against *Cryptococcus* and *Saccharomyces*. It seems that by increasing the chain length, the activity in this group increases. Addition of one or more polar groups to the alkyl chain reduces the antifungal activity (compare **6a**, **6c**, and **7** with **5b** and **5c**). Ionization of the functional group might be a negative factor in the observed bioactivity. Comparison of the activity of **5b** and **5c** with that of **7** or **6c**, which contain more amine groups per molecule, rules out the possibility that their activity is because of the amine group or pH change of the micro-environment around or inside the fungal cell. In the series of **5a**–**c**, **6c** and **7** the antifungal activities can be correlated with the lipophilicity of these molecules. Substituting one of the amine groups in **6c** with carboxyl to make **6a** does not improve the activity. However, the protected derivative **6b** shows a much better antifungal profile than **6a** and **6c**. Compound **6a** is present as "zwitterion" in the media, and this may hinder its absorption into and distribution within the fungal cell. The activity found in **1c** is extended to the amide derivative **9a** and is improved in **9b**, but the increase in the chain length to **9c** does not correspond to more potency against all the fungi tested. The addition of either acidic or alkaline polar groups to the side chain of **9b**, like in **9d** and **9e**, reduces the overall activity again. Derivative

9g, which has bulky non-polar groups together with polar amid groups, is inactive to all the tested fungi.

Amino acid derivatives of cinnamic acid, which is biosynthetically related to coumarins, have been synthesized and shown antifungal activity [9]. Their activity has been attributed to a better transport ability into the fungal cell. It has been discussed that an oligopeptide transporter system presents in fungal cell that helps transfer of molecules into the cell. This phenomenon has been described for *Bacillus subtilis* antifungal toxins as well [10]. Therefore, it was decided that the amino acid derivatives of angelicin be synthesized and tested for their antifungal activity. This group of compounds is totally inactive against *Candida*, however, contrary to many other tested compounds in this report, **8d** and **8f** show moderate activity against *Aspergillus*. Although an earlier work [11] suggested that an unprotected Leu-coumarin derivative could be the strongest antifungal in the coumarin series, the furanocoumarins containing amino acids here show a preferred activity while carrying a Phe group. The general MIC values of this group are not considered strong, however they show improvement compared to compound **1b**. This superior activity might be due to simple transformation of carboxyl group of **1b** to a more non-polar moiety. However, the more polar derivatives **8b**, **8d**, and **8f** exhibit improvement in their antifungal activity in many cases, and this may indicate the involvement of other factors.

Experimental

1. Chemistry

The preparation of compounds **1**–**9** has been reported elsewhere [6].

2. Antifungal susceptibility test

The antifungal activity was measured based on the recommendations of NCCLS [12]. The compounds were dissolved in acetone and diluted in a twofold manner in RPMI 1640 (pH = 7.0) in 96 microwell plates. The MIC was the minimum concentration of the agent that shows a full inhibition of the fungal growth in the well, examined by naked eyes.

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Essential oil volatiles of the roots of *Dorstenia psilurus* from Cameroon

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Dorstenia psilurus (Moraceae) is a small herb reaching 80 cm in height. It grows in the tropical rain forest zone in West, East and Central Africa, whereas it is used especially in traditional medicine: a decoction of leaves and roots is used to treat rheumatism, snake-bites, headache and stomach disorders [1], but no data on the essential oil volatiles of the roots of this *Dorstenia* species were given until now.

The essential oil was olfactorically evaluated by professional perfumers and the odor described as follows: spicy, terpinene- and pinene-like with fresh (limonene) side notes.

Using GC/FID and GC/MS in combination with olfactoric methods 24 components could be identified (see Table; unknown 1.8%).

Table: Essential oil volatiles of the roots of *Dorstenia psilurus* from Cameroon

Compound[a]	%[b]	RI[c]
1-Hexen-3-ol	0.3	773
α-Pinene	8.3	941
Camphene	1.1	953
1-Octen-3-ol	0.8	970
Sabinene	15.1	974
β-Pinene	7.5	980
para-Cymene	5.5	1012
Limonene	5.2	1023
cis-β-Ocimene	1.3	1028
β-Phellandrene	5.9	1031
γ-Terpinene	11.3	1055
Terpinolene	9.7	1073
Nonanal	0.4	1082
Camphor	0.6	1121
Terpinen-4-ol	1.2	1157
α-Terpineol	0.8	1163
β-Cubebene	2.1	1382
trans-β-Bergamotene	0.3	1422
β-Caryophyllene	4.2	1426
β-Gurjunene	6.5	1430
trans-β-Farnesene	1.0	1445
α-Humulene	3.8	1452
δ-Cadinene	5.3	1502

[a] in order of their retention times
[b] calculated as %-peak area of GC/FID analysis
[c] retention indices on unpolar column

As main compounds especially monoterpenes, sesquiterpenes and minor aliphatic derivatives were found. The identified terpinene- and pinene-derivatives as well as limonene are responsible for the characteristic odor of this essential oil.

In correlation to published data [2–8] on the bioactivity of volatiles, monoterpene hydrocarbons (like pinenes and terpinenes) show spasmogenic (on the ileum of pigs) and stimulating (found by special brain wave pattern changes) effects. Antiseptic, bactericidal and fungicidal activity can be attributed to mono- (e.g. pinenes and terpinenes) and sesquiterpenes (e.g. caryophyllene). Camphene-, pinene-