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FOUR NEW COUMARINS FROM THE ROOTS OF *PEUCEDANUM ARENARIUM**

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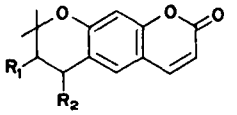
Key Word Index—*Peucedanum arenarium* var. *arenarium*; Umbelliferae; natural coumarins; peuarin; peuchlorin; peuchlorinin; peuchloridin.

Abstract—The roots of *Peucedanum arenarium var. arenarium* revealed the presence of 4 new natural coumarins. By spectral data their structures were elucidated as follows: peuarin, 3'-angeloyl-4'-methylisokhellactone; peuchlorin, 3'-angeloyl-4'-(2''-hydroxy-2''-methyl-3''-chloro)-butyroylisokhellactone; peuchlorinin, 3'-(2''-methylepoxy)-butyroyl-4'-(2''-hydroxy-2''-methyl-3''-chloro)-butyroylisokhellactone; peucloridin 3',4'-di-(2''-hydroxy-2''-methyl-3''-chloro)-butyroylisokhellactone.

INTRODUCTION

In previous investigations [1–3], the presence of bergapten, xanthalin (**1**), and two new dihydro-pyranocoumarins peuarenarine (**2**) and peuarenine (**3**) in the roots, and umbelliprenin, xanthalin, peuarenarine and peuarenine in the fruits of Bulgarian umbellifer *Peucedanum arenarium var. arenarium* has been reported.



A = OCOCMe=CHMe
B = OCOCMe(OH)CHMeCl
C = OCOCMe—CHMe
O

(1) Xantholin	$R_1 = R_2 = A$
(2) Peuarenarine	$R_1 = A$, $R_2 = C$
(3) Peuarenine	$R_1 = R_2 = C$
(4) Peuarin	$R_1 = A$, $R_2 = OMe$
(5) Peuchlorin	$R_1 = A$, $R_2 = B$
(6) Peuchlorinin	$R_1 = C$, $R_2 = B$
(7) Peuchloridin	$R_1 = R_2 = B$
(8) *cis* - Isokhellactone	$R_1 = R_2 = OH$

RESULTS AND DISCUSSION

Examination of the extractives of the roots of this plant has been extended and we now report the isolation of four additional minor extractives. All 4 products have nearly identical UV spectra with five maxima. According to Nielsen [4], they are pyranocoumarins having oxygen atoms in both the 3' and 4' positions. IR spectra have shown features, typical for coumarin system and besides, these of products (**6**) and (**7**) have shown an absorption at 3540 cm^{-1} for a hydroxyl group. NMR spectra of all four products showed signals typical for 3',4'dihydropyrano-5',6':6,7-coumarins: (i) an AB system which could be assigned to H-4 and H-3 of a coumarin grouping (δ_A 7·52–7·59, δ_B 6·21–6·24, J_{AB} 10 Hz), (ii) 2 singlets assinged to 2 *para*-related aromatic protons H-5 and H-8 (7·28–7·44 and 6·76–6·80), (iii) an AB system which could be assigned to H-4' and H-3' of the dihydropyran ring (δ_A 6·29–6·36 and δ_B 5·45–5·48, J_{AB} 4 Hz in the case of coumarins (**5**) and (**6**) and δ_A 4·35 and δ_B 5·33, J_{AB} 5 Hz in the case of coumarin (**4**); in the case of coumarin (**7**) these values are shifted to 6·64 and 5·82 because of the influence of the solvent. The last fact was obvious comparing the spectra of coumarin (**6**) in $CDCl_3$ and C_5D_5N); (iv) singlet or very close singlets assigned to 2'-gem-dimethyls.

The physical and spectral data of these extractives indicated that they are new natural coumarins, for which we propose the trivial names of peuarin (**4**), peuchlorin (**5**), peuchlorinin (**6**), and peuchloridin (**7**).

Coumarin (**4**), peuarin, $C_{20}H_{22}O_6$, mp 85·5–86·5°, showed in its NMR spectrum a singlet at δ 3·54 (3-H) assigned to OCH_3 group and a pattern of signals, characteristic for an angelic acid moiety (A). Comparison of this natural product with synthetic 3'-angeloyl-4'-methylisokhellactone [2] showed they were identical.

The NMR spectrum of peuchlorin (**5**), $C_{24}H_{27}O_8Cl$, mp 88–89°, besides the signals of 3',4'-dihydroxanthyletin skeleton, revealed the presence of 2 acyl groups. The broad multiplet at δ 6·15 (1-H) and the overlapped singlet and doublet at δ 1·88 (6-H) were indicative for angelic acid moiety (A). The AB_3 system of a quartet at δ_A 4·40 (1-H) and a doublet at δ_B 1·58 (3-H, J_{AB} 7 Hz) and the singlet at δ 1·47 (3-H) showed the probable structure of the second acid moiety to be B.

The NMR spectrum of the third coumarin, peuchlorinin (**6**), $C_{24}H_{27}O_9Cl$, mp 193–194°, revealed the presence of the acids B and C as acyl components. The acid B was obvious by a singlet at δ 1·48 (3-H) and a quartet at δ 4·42 (1-H, J 6·5 Hz) and a doublet at δ 1·58 (3-H, J 6·5 Hz), and the acid C with a quartet at δ 3·01 (1-H, J 5·5 Hz), a doublet at δ 1·27 (3-H, J 5·5 Hz: and a singlet at δ 1·40 (3-H). The presence of the hydroxyl hydrogen was obvious with a singlet at δ 3·04 which disappeared after exchange with D_2O.

The NMR spectrum of coumarin (**7**), peuchloridin, $C_{24}H_{28}O_9Cl_2$, mp 174–176° showed the presence of 2

* Part 8 in the series "Natural Coumarins". For Part 7 see Soine T. O., Zheleva A. B., Mahandru M. M., Erhard P., Bubeva-Ivanova L. (1973) *J. Pharm. Sci.* **62**, 1879.

moieties of the acid B: 2 quartets at δ 4·80 (1-H, J 7 Hz) and 4·60 (1-H, J 7 Hz) and the corresponding doublets at 1·65 (3-H, J 7 Hz) and 1·57 (1-H, (1-H, J 7 Hz), as well as 2 singlets of isolated methyls at 1·65 (3-H) and 1·46 (3-H). After irradiating the signal at δ 4·60 the doublet at δ 1·57 collapsed to singlet at δ 1·57 and an irradiation of the quartet at 4·80 led to collapse of the doublet at 1·65.

The exact positions of the acids in (**5**) and (**6**) were revealed by MS spectra of these products. According to Zaharov *et al.* [5] the substituent at 3'-position is lost mainly as RCOOH and much less as RCOO, while the 4'-substituent shows the main loss of RCOO and much less as RCOOH. Indeed, the spectrum of (**2**), a linear coumarin with different acid moieties in positions 3' and 4', unambiguously established by mild alkaline hydrolysis, confirmed this. In the case of (**2**) the abundance of M^+-R^3'COOH (*m/e* 342) was 20%, while that of M^+-R^3'COO (*m/e* 343) was 7%. The substituent at 4'-position showed M^+-R^4'COO (*m/e* 327) in the abundance of 40% and M^+-R^4'COOH (*m/e* 326)—4%.

In the mass spectrum of (**5**) M^+-RCOOH for the angeloyl component was more abundant than M^+-RCOO (*m/e* 378, 3%; *m/e* of M^+-RCOO -null), which determined the 3'-position of the angeloyl moiety. At the same time M^+-RCOO for B acid (*m/e* 327) showed 20% abundance, while M^+-RCOOH (*m/e* 326) revealed 12%, which confirmed the 4' position for B acid.

In the case of coumarin (**6**) the corresponding values were as follows: *m/e* of M^+-R^3'COOH (378)—6%, M^+-R^3'COO (*m/e* 379)—null, *m/e* of M^+-R^4'COO (343)–40%, *m/e* of M^+-R^4'COOH (342)–null. This confirmed the proposed 3' position for the acyl C and 4' for the acyl B.

The coupling constants of the protons at the 3' and 4' positions of the 3 coumarin diesters (**5**, **6** and **7**) -4 Hz as well as the values of the signals from gem-dimethyls showed that the novel natural coumarins are *cis*-products [3,4] as are the previous ones (**1**–**3**).

Chlorine-containing pyranocoumarins have not been isolated until now and the only chlorine-containing furanocoumarin saxalin [6,7] has been considered by Avramenko and Nikonov [6] as an original product of *Angelica saxatilis* while Ignat'eva *et al.* [7] have reported that saxalin isolated by them from *Cachrys pubescens* was an artifcat. The natural origin of the newly isolated compounds (including the chlorine-containing ones) has been established by TLC of C_6H_6, Et_2O, $CHCl_3$, acetone and methanol extracts of the plant material (without any additional treatment) in different systems and mass spectrometry of the above-mentioned extracts.

EXPERIMENTAL

The plant material was gathered and identified by P. Savtchev and A. Mytrev, Chemical Pharmaceutical Research Institute, Sofia-56, Bulgaria. Authentic specimens of the plant have been placed in the Herbarium of Chemical Pharmaceutical Research Institute, Sofia-56, Bulgaria.

Mp's are uncorrected. Unless otherwise stated, UV spectra were measured in EtOH, IR spectra in $CHCl_3$ and 60 and 100 MHz NMR spectra—in $CDCl_3$ (tetramethylsilane as internal reference). Only significant bands from these spectra are quoted. MS were determined using a source temperature of 150° and ionizing voltage of 70 eV. Evaporation refers to evaporation under diminished pres. TLC separations were carried on plates using Merck Kieselgel G. and -*A*-C_6H_6-toluene-EtOAc-HCO_2H (10:5:4:1); *B*-$CHCl_3$-Me_2CO (9:1). When substances are stated to be identical then this has been established by comparison of mp's, UV, IR, NMR and MS spectra and TLC behavior. *Peuarin* (**4**) 40 kg of ground roots were extracted with MeOH (1:5, 3 ×) at room temp. Extracts were collected, filtered and evaporated. Column chromatography on neutral alumina led to isolation of pure (**1**), (**2**), (**3**) [1–3] and mixtures A, B, and C. Mixture A (3·7 g) showed on TLC plate the presence of xanthalin (R_f 0·7, system A) and second product (R_f 0·6) 0·5 g of this mixture were separated by preparative TLC to give 0·2 g of (**4**), mp 85·5–86·5°, $[\alpha]_D^{25}$ −87·3° (*c* 0·30, chloroform) Found: C, 66·85; H, 6·29, M (mass spectrum) 358, $C_{20}H_{26}O_6$ requires: C, 67·03; H, 6·14%. UV: λ_{max} 222 (log ϵ 4·45), 247 *sh* (3·69), 258 (3·59), 300 *sh* (409), and 325 (4·28). Peuchlorin and peuchlorinin. Mixture B (see above) showed on TLC plate (in the system A) the presence of 2 spots with violet fluorescence and R_fs 0·50 and 0·30. After preparative TLC of this mixture (0·5 g) (**5**) (0·0156 g, R_f 0·50) and (**6**) (0·3 g) were obtained.

Peuchlorin. mp 88–89°, UV: λ_{max} 220 (4·20), 246 (3·52), 257 (3·55), 300 *sh* (4·08), 324 (4·30) nm. High resolution mass spectrum: 478·1394, possible constitutional formulae $C_{24}H_{27}O_8Cl$.

Peuchlorinin (**6**) mp 193–194°, $[\alpha]_D^{25}$ + 6·6° (*c* 0·44, $CHCl_3$). Found: C, 58·30; H, 5·76, Cl, 7·42, M (MS)494, $C_{24}H_{27}O_9Cl$ requires: C, 58·24; H, 5·46; Cl, 7·1%. UV: λ_{max} 220 (log ϵ 4·18), 246 (3·50), 257 (3·47), 300 *sh* (4·06), 324 (4·27) nm.

Peuchloridin. Besides (**6**) mixture C (see above) showed the presence of second spot on TLC plates R_f of peuchlorinin 0·45, (R_f of the second spot 0·37, system B). Fractionation by preparative TLC gave peuchlorinin and the second compound, peuchloridin (**7**), mp 174–176°, $[\alpha]_D^{25}$ −49·4° (*c* 0·30, methanol) Found: C, 54·48; H, 5·29; Cl, 13·31%. M (MS) 530, $C_{24}H_{28}O_9Cl_2$ requires: C, 54·36; H, 5·28; Cl, 13·20%. UV: λ_{max} 220 (log ϵ 4·28), 246 (3·60), 257 (3·60), 300 *sh* (4·20), 324 (4·38) nm.

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