

Natural Coumarins V: Isolation of Xanthalin and a New Pyranocoumarin, Peuarenine, from *Peucedanum arenarium* W.K.

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Abstract □ Identification of xanthalin (3',4'-diangeloxy-3',4'-dihydroxanthyletin) as one of the three coumarins obtained from *Peucedanum arenarium* W.K. is provided by direct comparison. Peuarenarine, m.p. 114–116°, and peuarenine, m.p. 192–194°, have been shown to be diesters corresponding to 3'-angeloxy-4'-2,3-epoxy-2-methylbutyryloxy-3',4'-dihydroxanthyletin and 3',4'-di-2,3-epoxy-2-methylbutyryloxy-3',4'-dihydroxanthyletin, respectively, by spectral and chemical procedures. A *cis*-relationship of the two adjacent acyloxy functions in all three cases is suggested on the basis of previous NMR studies by others on closely related compounds.

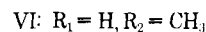
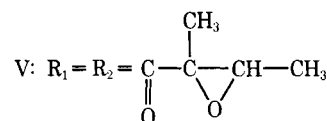
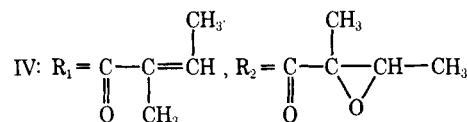
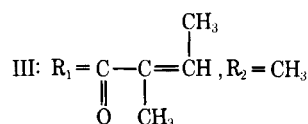
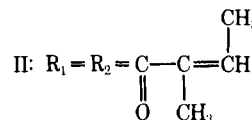
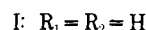
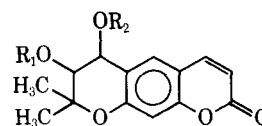
Keyphrases □ *Peucedanum arenarium*—isolation of xanthalin and peuarenine □ Xanthalin—isolated from *Peucedanum arenarium* □ Peuarenine—isolated from *Peucedanum arenarium* □ Coumarins, natural— isolation of xanthalin and peuarenine from *Peucedanum arenarium*

Previously (1, 2), it was reported that three diesters of isokhellactone (I) (3',4'-dihydroxy-3',4'-dihydroxanthyletin) were isolated from fruits and roots of *Peucedanum arenarium* W.K.¹

DISCUSSION

One of these (coumarin 3, m.p. 113–115°) showed the presence of two angelate moieties [*i.e.*, two doublets at δ 1.85 and 2.07 (6H each) and a broad multiplet at δ 6.10 (2H)] in its NMR spectrum and has been identified as xanthalin (II), first isolated in 1968 from *Xanthogalum purpureum* by Sokolova *et al.* (3), through mixed melting point, UV, IR, and NMR spectral comparisons with an authentic sample. The previously observed discrepancy in specific rotation $\{[\alpha]_D^{25} - 92^\circ$ (c 0.97, ethanol) for coumarin 3 and $[\alpha]_D^{20} - 164.2^\circ$ (c 0.97, ethanol) for II (4)¹ appears to be due to instrumental differences². A similar consideration, together with differing solvents, undoubtedly accounts for the observed differences in specific rotations of the saponification products of the two substances in dioxane [the diol obtained from II was reported (4) to have m.p. 213–215°, $[\alpha]_D^{20} 0^\circ$ (c 0.13, ethanol), whereas the product obtained from coumarin 3 under the same conditions has m.p. 217–219°, $[\alpha]_D^{25} - 21.5^\circ$ (c 0.20, pyridine)]. The identity of coumarin 3 with II, therefore, seems well established.

Peuarenarine [coumarin 1, m.p. 114–116° (2)] was obviously new; and while the respective placements of the two acyl components on the isokhellactone skeleton had not been established, it was known that these moieties were derived from angelic acid and 2,3-epoxy-2-methylbutyric acid, respectively. The present work takes advantage of the well-known facts (5–8) that the ester moiety at the benzylic 4'-position is more labile than the 3'-ester moiety and that it undergoes eventual replacement by an alkoxy group derived from the alcoholic fixed base employed for saponification. Thus, a 20-sec. saponification of peuarenarine in 0.5 N methanolic KOH provided,



via chromatography on acidic alumina, a product, $\text{C}_{20}\text{H}_{22}\text{O}_6$, m.p. 83–85°, $[\alpha]_D^{25} - 83.5^\circ$ (c 0.28, CHCl_3). The NMR spectrum of this product showed the usual multiplet pattern and integration for the vinyl and methyl protons of a *single* angelate moiety and a signal at δ 3.60 (3H), characteristic of the OCH_3 group. Experiments with II under the same conditions provided a substance that was identical to that obtained from peuarenarine; therefore, the structure of the latter is correctly represented as IV.

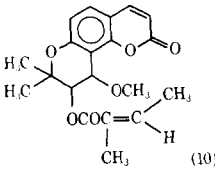
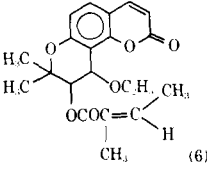
The third coumarin [coumarin 2, m.p. 192–194°, $[\alpha]_D^{25} + 51.12^\circ$ (c 0.26, CHCl_3)] showed the common nuclear and gem-dimethyl group NMR spectral characteristics of the isokhellactone skeleton except for the signals attributable to the two acyl groups. The latter signals indicated through their character and intensity that two 2,3-epoxy-2-methylbutyric acid moieties were present. By using pyridine as a solvent, two doublets at δ 1.27 and 1.52 (3H each, $J = 5.5$ Hz.) due to methyl groups adjacent to a proton were observed. Quartets, arising from the methine protons, overlapped to give a quintet (2H) at δ 3.07 ($J = 5.5$ Hz.). Besides these signals, singlets at δ 1.68 (3H) and 1.49 (3H), characteristic of the two isolated methyl groups, were observed. This new compound, for which the name "peuarenine" is proposed, was thus shown to be the diester (V) of isokhellactone and 2,3-epoxy-2-methylbutyric acid. The coupling constants of the protons at the 3'- and 4'-positions ($J = 4$ Hz.) of the three coumarins under consideration indicate that they are *cis*-diesters, since the studies of El-Antably (9) showed that synthetic *cis*-diesters of isokhellactone have $J = 4$ Hz. and *trans*-diesters have $J = 6$ Hz., regardless of the character of the acylating moiety.

The configurational assignment of the glycol obtained by saponification was also arrived at through the El-Antably study (9), even though there is the distinct possibility of epimerization at the 4'-position based on the mechanism of the reaction. As a consequence, even though the diester being saponified was *cis*, the diol obtained by the usual isolation techniques could well be the one least soluble (or more easily crystallized) and the other could be disposed of, inadvertently, in the mother liquors. The synthetic diols showed a wide

¹ This plant material was gathered and identified by P. Savtchev and A. Mytrev, Chemical Pharmaceutical Research Institute, Sofia, Bulgaria, the collection being made on the seaside sands of the Bulgarian coast. The plant is also designated as *P. arenarium* var. *arenarium* and is endemic to the Balkan peninsula, being found only in Albania, Yugoslavia, and Bulgaria. "Flora Europaea," T. G. Tutin and W. H. Heywood, Eds., Cambridge, England, 1968, p. 362; "Flora Bulgarica," part II, N. Stojanov, B. Stefanov, and B. Kitanov, Eds., Sofia, Bulgaria, 1967, p. 804.

² When measured on a "Roussel-Jouan" electron polarimeter in chloroform, the specific rotations were $[\alpha]_D^{25} - 92.5^\circ$ (c 0.24) for II and $[\alpha]_D^{25} - 93.1^\circ$ (c 0.26) for coumarin 3.

Table I—NMR Signals of 3'- and 4'-Protons^a

	II	IV	V	I in Pyridine	III	VI in Benzene	VI in Pyridine		Methyl- khellac- tone (11)	
H ₃ '	5.53	5.41	5.41	4.05	5.38	3.86 (8 Hz.)	3.88 (8 Hz.)	5.27	3.51 (3.5 Hz.)	5.20 (2 Hz.)
H ₄ '	6.35	6.31	6.31	5.17	4.41	4.37	4.48	4.49	4.56	4.50

^a Unless otherwise stated, the spectra were determined in CDCl₃. Unless otherwise stated, $J_{3',4'}$ = 4 Hz. Figures for chemical shifts are in δ units.

variation, in dimethyl sulfoxide-*d*₆, in coupling constant for the *cis*-forms (J = 4 Hz.) and *trans*-forms (J = 9 Hz.); therefore, the diol isolated in these studies (2) is apparently the *cis*-diol.

The coupling constant for the 3'- and 4'-protons of the well-known (4) *trans*-methylisokhellactone (VI) isolated following saponification of peuarenine with methanolic fixed base for 5 min. was 8 Hz.

The chemical shifts for the 3'- and 4'-protons of the compounds reported herein (Table I) are in complete accord with those reported in the literature for the angular isomeric coumarins (6, 10, 11)³. The mass spectra of the three coumarins provided molecular ions supporting the structural assignments, and the fragmentation patterns were quite similar to those of related compounds (10, 13).

This work on the fruits and roots of *P. arenarium* W.K. shows that certain biogenetically related coumarin derivatives, representing basically diesters of the isokhellactone structure, are linear isomers of the corresponding angular pyranocoumarins isolated from *Laserpitium archangelica* Wulf. by Bohlmann and Thefeld (10). It is probable that the isolation of additional new coumarins of the linear series will occur in the future and will parallel similar developments in the discovery of new coumarins in the angular series derived from khellactone (*i.e.*, 3',4'-dihydroxy-3',4'-dihydroxyseselin).

EXPERIMENTAL⁴

Treatment of Xanthalin with Methanolic Potassium Hydroxide—A solution of xanthalin (1 g.) in methanol (200 ml.) was mixed with 200 ml. of 1 *N* methanolic potassium hydroxide, left at room temperature for 2 min., and then acidified with 1 *N* sulfuric acid. Upon standing for 45 min., 1000 ml. of water was added and the mixture was extracted thoroughly with chloroform. The chloroform extract was dried with anhydrous sodium sulfate and evaporated; the residue was chromatographed on a column of acid alumina (15 g.), with 100-ml. fractions of petroleum ether eluant being collected. Fractions 1–6 yielded 0.20 g. of 3'-angeloyl-4'-methoxy-3',4'-dihydroxanthyletin, m.p. 82–85°, $[\alpha]_D^{23}$ –84.13° (c 0.21, CHCl₃).

Treatment of Peuarenarine with Methanolic Potassium Hydroxide—A solution of peuarenarine (3 g.) in methanol (500 ml.)

was mixed with 300 ml. of 0.5 *N* potassium hydroxide and left at room temperature for 20 sec. The reaction mixture was acidified with 1 *N* sulfuric acid, allowed to stand for 45 min., and then diluted with 1000 ml. of water. The diluted mixture was extracted with chloroform, and the chloroform extract was dried (anhydrous sodium sulfate); the residue from evaporation of the chloroform was chromatographed on acid alumina (30 g.), using petroleum ether as the eluant and collecting 100-ml. fractions. Fractions 1–8 yielded 0.81 g. of a product, m.p. 83–85°, $[\alpha]_D^{23}$ –83.5° (c 0.28, CHCl₃). A mixed melting point with the product from the previous experiment failed to show depression.

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³ The principal exception occurs in the publication by Bohlmann *et al.* (12) which indicated an inverse relationship for Compounds 2, 3, 4, and 8 cited in a table in the paper. This apparently erroneous assignment was observed and corrected by M. E. Perel'son, Y. N. Sheinker, A. A. Savina, and G. P. Syrova, *Khim. Prir. Soedin.*, **1971**, 712.

⁴ Melting points were determined on a Boëtius micromelting-point apparatus checked for accuracy against a set of standard samples. IR spectra were determined in mineral oil on a UR-10 spectrophotometer, and UV spectra were determined on a Unicam SP-700 spectrophotometer. NMR spectra were determined on a JOEL JNM-C-605 (60 MHz.) spectrometer. Values of $[\alpha]_D$ were determined on an Electron Polarimeter "Roussel-Jouan." Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6D spectrometer. The source temperature was 250°, except for xanthalin which required 150°, and the ionizing voltage in all cases was 70 ev. "Acid alumina" refers to aluminum oxide for chromatographic adsorption analysis corresponding to Brockmann (II) acid.