Natural Coumarins VII: Isolation and Structure of a New Coumarin, Peuruthenicin, from *Peucedanum ruthenicum* M.B.

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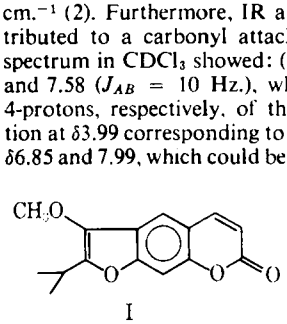
Abstract □ The roots of the Bulgarian umbellifer, *Peucedanum ruthenicum* M.B., yielded a novel naturally occurring coumarin, which was assigned the trivial name of peuruthenicin. It was identified as 6-carbomethoxy-7-hydroxycoumarin through both spectral (mass, NMR, IR, and UV) and chemical (degradative and synthetic) evidence. The latter consisted of alkaline hydrolysis to known umbelliferone-6-carboxylic acid (IV) and synthesis of peuruthenicin by methyl esterification of IV which had been obtained by chromate oxidation of marmesin.

Keyphrases □ Peuruthenicin (6-carbomethoxy-7-hydroxycoumarin)—isolation from *Peucedanum ruthenicum* M.B., structure determination □ Coumarins, natural—isolation of peuruthenicin (6-carbomethoxy-7-hydroxycoumarin) from *Peucedanum ruthenicum* M.B., structure determination □ 6-Carbomethoxy-7-hydroxycoumarin (peuruthenicin)—isolation from *Peucedanum ruthenicum* M.B., structure determination □ *Peucedanum ruthenicum* M.B.—isolation and structure identification of peuruthenicin (6-carbomethoxy-7-hydroxycoumarin)

Previously (1), a phytochemical examination of *Peucedanum ruthenicum* M.B., a native Bulgarian umbellifer, showed the presence of the furanocoumarin peucedanin (I) in the roots and the flavonol glycoside rutin in the flowers. Further examination of the root extract provided a new compound, assigned the trivial name peuruthenicin, which has been structurally characterized.

DISCUSSION

Peuruthenicin was obtained as cream-colored needles, m.p. 196–197°, for which the molecular ion derived from the mass spectrum and the supporting elemental analyses suggested the molecular formula $C_{11}H_8O_5$. Its UV spectrum suggested a possible coumarin chromophore. The IR spectrum was also compatible with a coumarin-type structure, since it showed a typical carbonyl absorption at 1740 cm.$^{-1}$ and the typical 3,4-double bond absorption at 1630 cm.$^{-1}$ (2). Furthermore, IR absorption at 1680 cm.$^{-1}$ could be attributed to a carbonyl attached to an aromatic ring. The NMR spectrum in $CDCl_3$ showed: (*a*) an *AB* system of doublets at δ6.25 and 7.58 (J_{AB} = 10 Hz.), which could be assigned to the 3- and 4-protons, respectively, of the coumarin nucleus; (*b*) an absorption at δ3.99 corresponding to a methoxyl group; (*c*) two singlets at δ6.85 and 7.99, which could be assigned to two *para*-related aromatic protons; and (*d*) a low field singlet at δ11.08, which could be assigned to chelated hydroxyl. The mass spectrum (Scheme I) indicated the facile loss of methanol from the molecular ion to provide a metastable ion (*m/e* 160.85), supported base peak at *m/e* 188. This behavior is well known for *ortho*-substituted methyl benzoates, particularly for the methyl salicylate type of moiety (3). Therefore, on the basis of the assembled spectral and analytical data, two possible structures could be entertained for peuruthenicin, namely II and III.



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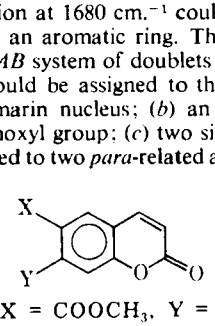
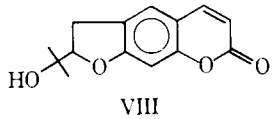


II: X = $COOCH_3$, Y = OH
III: X = OH, Y = $COOCH_3$
IV: X = COOH, Y = OH
V: X = H, Y = OH
VI: X = $COOCH_3$, Y = OCH_3
VII: X = COOH, Y = OCH_3



VIII

Alkaline hydrolysis of peuruthenicin gave a carboxylic acid, m.p. 258° dec., which failed to depress the melting point of an authentic sample of umbelliferone-6-carboxylic acid (IV) prepared by chromate oxidation of marmesin (VIII) as described previously (4). This acid also provided umbelliferone (V) when thermally decarboxylated to locate firmly the site of the phenolic hydroxyl group. Peuruthenicin, on treatment with diazomethane, provided a methyl ether, m.p. 168°, which was apparently identical to 6-carbomethoxy-7-methoxycoumarin (VI) (4).

The physical and chemical data strongly suggested that peuruthenicin was a novel naturally occurring coumarin with Structure II rather than III. This structure was validated by esterification of IV with methanolic sulfuric acid to give the methyl ester (II), which proved to be identical in all respects (UV, IR, NMR, and mass spectrum) with peuruthenicin.

Furthermore, VI was subjected to alkaline hydrolysis to provide VII, m.p. 237–239°, isomeric with II. Its NMR spectrum was distinctly different from that of II and showed two doublets at δ6.30 and 7.70 ($J_{3,4}$ = 10 Hz.) characteristic of the protons at the 3- and 4-positions, two *para*-related aromatic protons as singlets at δ6.91 and 8.23, and a methoxyl singlet at δ3.83.

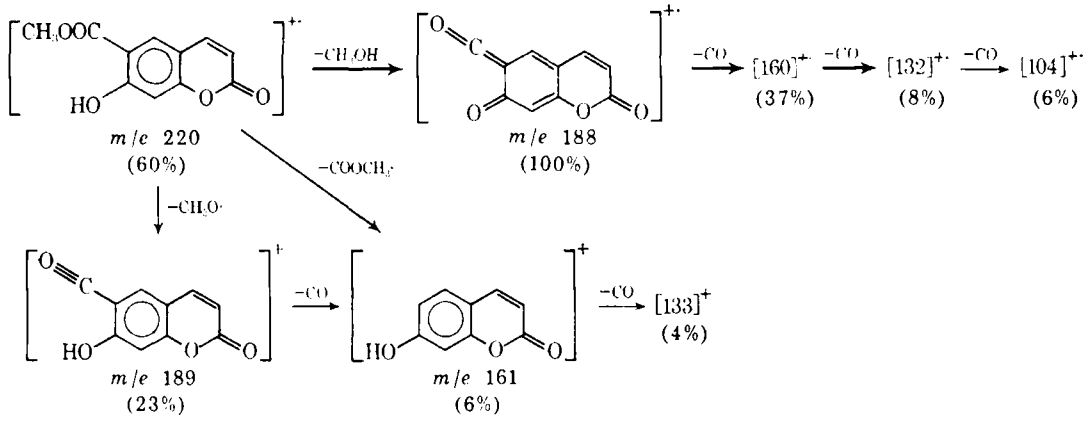
Although the present study was not concerned with the biosynthesis of peuruthenicin, it may well be that it is derived through a bio-oxidative transformation of peucedanin (I) or the corresponding ketone, oreoselone. Studies presently underway are exploring this possibility.

EXPERIMENTAL[1]

Isolation of Peuruthenicin—One kilogram of the roots of *P. ruthenicum* M.B., ground to a No. 20 powder, was extracted with methanol at room temperature using successively 10, 8, and 6 l. of solvent[2]. The extract was then filtered until clear and evaporated to give a gummy residue. This residue, when treated with 1 l. of water, gave a cloudy suspension which was extracted with ether (3 × 300

[1] Melting points were determined using a Reichert hot-stage apparatus and are uncorrected. Unless otherwise stated, IR spectra were measured in chloroform on a Perkin-Elmer 157G grating spectrophotometer, UV spectra were measured in 95% ethanol on a Cary 14 spectrophotometer, and 60- and 100-MHz. NMR spectra were measured in $CDCl_3$ (tetramethylsilane as internal standard) on HA 100 or R 12 NMR spectrometers. Only significant bands from these spectra are cited. Mass spectra (Scheme I) were determined using an AEI-MS 12 mass spectrometer as well as a Hitachi Perkin-Elmer RMU-6D instrument; in each case the source temperature was 150° and the ionizing voltage was 70 ev. Elemental analyses were obtained from M-H-W Laboratories, Garden City, Mich. Evaporation refers to evaporation under reduced pressure. TLC separations were carried out on plates coated with Merck kieselgel G and a solvent system consisting of benzene–toluene–ethyl acetate–formic acid (10:5:4:1). The drying agent for ethereal solutions was anhydrous sodium sulfate, and substances stated to be identical were thus established by comparison of melting points; IR, NMR, and mass spectra; and TLC behavior.

[2] That peuruthenicin was not an artifact formed by possible esterification of umbelliferone-6-carboxylic acid because of the methanol solvent was shown by extraction of the original plant material with several different solvents: benzene, petroleum ether, ether, chloroform, acetone, ethanol, and methanol. Each extract, without further treatment, was spotted on a TLC plate and developed with the benzene–toluene–ethyl acetate–formic acid solvent described. There was no doubt that peuruthenicin was the original coumarin in each extract since the R_f value of peuruthenicin is 0.60 and that of the acid is 0.4.



Scheme I—Mass spectral fragmentation of peuruthenicin. Heavy arrows indicate that the proposed fragmentation pathway is supported by the appearance of a metastable ion.

ml.). The combined ethereal extracts were dried and evaporated, and the residue was successively extracted with 200-ml. portions of boiling petroleum ether (b.p. 40–60°). The extracts, in sequence, were examined by TLC, which revealed that the first 10 extracts contained mainly peucedanin but that the following five extracts contained a 1:1 mixture of peucedanin and an unknown coumarin (*i.e.*, peuruthenicin). The latter extracts were combined, concentrated to about 100 ml., and held at 0° for 12 hr., at which time a crystalline precipitate of peucedanin had been deposited. This was removed by filtration and the filtrate was reduced to a volume of 50 ml. and then kept at 0° for an additional 15 days, during which time an amorphous precipitate formed and was collected by filtration. Recrystallization of this precipitate from methanol gave peuruthenicin (1 g.) as cream-colored needles, m.p. 196–197°; UV $\lambda_{max}^{C_2H_5OH}$ (log ϵ): 218 (4.30), 224 (4.28), 245 (4.46), 292 (3.99, shoulder), 3.03 (4.06), 323 (4.16), and 332 nm. (4.15); IR ν_{max}: 1740, 1680, and 1630 cm.$^{-1}$; mass spectrometry: *m/e* 220, 189, 188, 161, 160, 133, 132, and 104.

Anal.—Calc. for $C_{11}H_8O_3$: C, 60.0; H, 3.6; M (mass spectrum), 220. Found: C, 60.1; H, 3.7; M, 220.

Alkaline Hydrolysis of Peuruthenicin—Peuruthenicin (200 mg.) was dissolved in dioxane (40 ml.) and treated with aqueous 20% sodium hydroxide (80 ml.) under reflux for 30 min. The cooled mixture was then diluted to approximately 500 ml. with water, acidified with diluted sulfuric acid, and extracted with chloroform. The chloroform extract was dried and evaporated to yield 80 mg. of white crystals, m.p. 258° dec., identical to umbelliferone-6-carboxylic acid (IV) as determined by melting point, mixed melting point, and spectral comparison with the oxidative product of marmesin.

Decarboxylation of Alkaline Hydrolysis Product—The preceding acid (50 mg.) was heated at 265° for 2 hr. under a sublimation unit; the collected sublimate was shown to have a melting point of 230° and was identical to authentic umbelliferone.

Methylation of Alkaline Hydrolysis Product—The preceding acid (59 mg.) was dissolved in acetone (5 ml.) and treated for 20 min. with excess ethereal diazomethane generated from *N*-nitrosomethylurea in the usual way (5). Evaporation of the reaction mixture and crystallization of the residue from methanol gave white crystals (60 mg.), m.p. 167° [lit. (4) m.p. 168°]; IR ν_{max}: 1740–1720 and 1622 cm.$^{-1}$; NMR: δ6.30 and 7.66 (doublets, *AB* system, J_{AB} = 10 Hz., 3-H and 4-H), 8.00 (singlet, 5-H), 6.87 (singlet, 8-H), and 3.97 and 3.90 (singlets, $COOCH_3$ and OCH_3).

Umbelliferone-6-carboxylic Acid (IV)—Marmesin (1 g.) was oxidized, according to a published method (4), with 3% sulfuric acid and potassium dichromate to yield umbelliferone-6-carboxylic acid (300 mg.), m.p. 259°, identical with the product of alkaline hydrolysis of peuruthenicin as shown previously.

Synthesis of Peuruthenicin—Umbelliferone-6-carboxylic acid (90 mg.), methanol (5 ml.) and concentrated sulfuric acid (0.1 ml.) were heated together for 48 hr. under reflux. The resulting mixture was cooled, neutralized with 5% aqueous potassium hydroxide, and diluted with water until no further precipitate formed. The precipitate was collected and fractionated by preparative TLC to give cream-colored crystals (47 mg.), m.p. 196°, identical with the peuruthenicin obtained from natural sources.

7-Methoxycoumarin-6-carboxylic Acid (VII)—Peuruthenicin methyl ether (VI) (50 mg.) was dissolved in acetone (2 ml.) and treated with 5% aqueous potassium hydroxide (2 ml.) at room temperature for 7 hr. and then heated under reflux for 15 min. The cooled mixture, upon acidification with diluted sulfuric acid and dilution with water, yielded a precipitate which was collected and subjected to preparative TLC separation. The principal product was removed from the plates by scraping and, when extracted with methanol, yielded a white crystalline material (35 mg.), m.p. 237–239°; IR ν_{max}: 1730 and 1630 cm.$^{-1}$; NMR (in pyridine): δ6.30 and 7.70 (doublets, *AB* system, J_{AB} = 10 Hz., 3-H and 4-H), 8.23 (singlet, 5-H), 6.91 (singlet, 8-H), and 3.83 (singlet, OCH_3).

REFERENCES

(1) L. Bubeva-Ivanova, A. Zheleva, and P. Savtchev, *Khim. Prir. Soedin.*, **5**, 594(1969); through *Chem. Abstr.*, **73**, 73827s (1970).

(2) T. O. Soine, *J. Pharm. Sci.*, **53**, 231(1964).

(3) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1964, pp. 194–199.

(4) A. Chatterjee and S. Mitra, *J. Amer. Chem. Soc.*, **71**, 606 (1948).

(5) W. E. Bachmann and W. S. Struve, in "Organic Reactions," vol. I, R. Adams, W. E. Bachmann, L. F. Fieser, J. R. Johnson, and H. R. Snyder, Eds., Wiley, New York, N. Y., 1942, p. 50.

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