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Coumarins IX: Coumarins of *Sphenosciadium capitellatum* (A. Gray)

KUO-HSIUNG LEE* and T. O. SOINE†

Abstract □ The aliphatic naphtha extract of the roots of *Sphenosciadium capitellatum* (A. Gray) has yielded isoimperatorin, phellopterin, imperatorin, oxypeucedanin, isopimpinellin, and a linear furanocoumarin, $C_{17}H_{16}O_5$, which has been assigned the 5-γ,γ-dimethylallyloxy-8-methoxy psoralen structure on the basis of degradative and spectral studies. This linear furanocoumarin appears to be identical to cnidilin, recently isolated and characterized from *Cnidium dubium* (Schkuhr) Thell. The methanol extract yielded sucrose and the glycosidic coumarin, nodakenin, in excellent yield.

Keyphrases □ Coumarins—*Sphenosciadium capitellatum* □ *S. capitellatum* roots—coumarin extraction □ Column chromatography—separation □ Paper chromatography—separation, identity □ IR spectrophotometry—structure, identity □ UV spectrophotometry—structure, identity □ NMR spectroscopy—structure, identity □ Mass spectroscopy—molecular weight

Examination of the coumarinic content of the roots of *Sphenosciadium capitellatum* (A. Gray) is a continuation of a general program (1–5) for investigating umbelliferous plants for agents with potentially useful physiological activity (6). Of further interest was the fact that this particular species is the sole representative of the *Sphenosciadium* genus. The extraction of the coumarins was carried out by using aliphatic naphtha,[1] ether, and methanol, respectively. The aliphatic naphtha extract was column-chromatographed over silica gel and yielded a number of known linear furanocoumarins, *i.e.*, isoimperatorin (I), phellopterin (II), imperatorin (III), oxypeucedanin (IV), and isopimpinellin (V). In addition, an apparently undescribed furanocoumarin (VI) was obtained.

The ethereal extract was not investigated further since it showed virtually identical components when compared by TLC with the aliphatic naphtha extract.

Compound VI, m.p. 115–115.5°, shows a molecular ion peak at m/e 300 in the mass spectrum and is optically inactive. Its elementary analysis agrees with a molecular formula of $C_{17}H_{16}O_5$ and it appears as a single fluorescent yellow spot under UV light by TLC on Silica gel G in three different solvent systems.[2] The IR spectrum indicated that VI is a furanocoumarin (7).

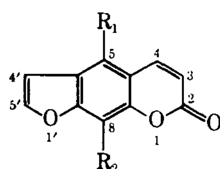
The UV spectrum of Compound VI is virtually superimposable upon that of phellopterin (II) (m.p. 101–102°) (7). It remained unchanged on the addition of sodium hydroxide, indicating the absence of a free phenolic hydroxy group. The possibilities for the existence of an angular system like that of either VII or VIII for the structure of Compound VI could be ruled out since an angular furanocoumarin would show a completely different UV spectrum compared with that of the corresponding linear one (7).

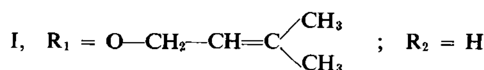
Compound VI displayed NMR signals at 8.33 (3H, s), 8.22 (3H, s) ($=C{<}^{C\underline{H}_3}_{C\underline{H}_3}$), 5.87 (3H, s, $-OC\underline{H}_3$), 5.21 (2H, d, J = 7 c.p.s., $-C\underline{H}_2-$), 4.44 (1H, t, J = 7 c.p.s., $-C\underline{H}=$), 3.74 (1H, d, $C_3-\underline{H}$, $J_{3,4}$ = 10 c.p.s.), 1.85 (1H, d, $C_4-\underline{H}$, $J_{3,4}$ = 10 c.p.s.), 3.07 (1H, d, C_4'-$\underline{H}$, $J_{4',5'}$ = 2 c.p.s.) and 2.38τ (1H, d, $C_5'-\underline{H}$, $J_{4',5'}$ = 2 c.p.s.) and is in fact also identical with that of phellopterin (II) except for the splitting of the gem-dimethyl group into a doublet instead of the singlet which is found in the latter (7).

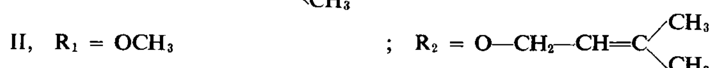
The assignment of a γ,γ-dimethylallyloxy group as the C_5-substituent in place of a γ,γ-dimethylallyl group was quite apparent from the evidences of its elementary

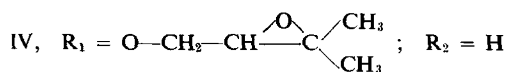
[1] Skellysolve B, Skelly Oil Co., Kansas City, Mo.

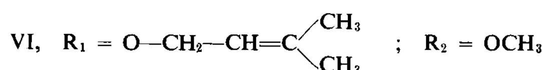
[2] *a*, Skellysolve B–ethyl acetate = 3:1; *b*, skellysolve B–benzene–methanol = 5:4:2; *c*, ethyl acetate–a mixture of 2:1 dichloromethane–carbon tetrachloride = 8:92.

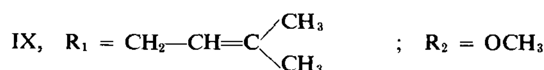


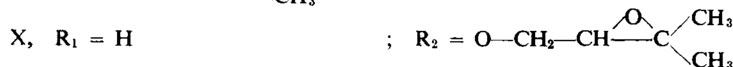


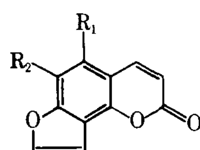


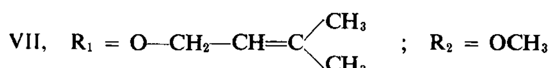


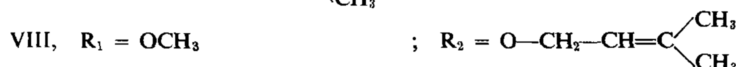


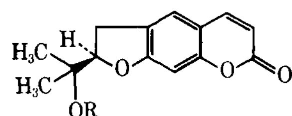


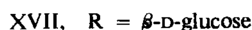












analysis, molecular weight determination by mass spectrometry (*m/e* 300), the characteristic ion of *m/e* 69 (22.8%) (q.v.) of the former and a different melting point than the latter, namely, the monomethyl ether of alloimperatorin (IX) (8), which showed m.p. 104–105°.

From the above evidence, Structure VI, the isomer of phellopterin (II), was proposed for this compound.

Considering the structural relationship of Compound VI and phellopterin, it might be expected that their mass spectral fragmentations would be quite similar and, indeed, they are almost identical in the mass peaks except for some differences in the abundance ratios. The mass spectrum of phellopterin had been determined previously by Vul'fson *et al.* (9) but no detailed fragmentation pathways were suggested.

The mass spectrum of phellopterin (see Fig. 1) showed a molecular ion peak at *m/e* 300 and a base peak at *m/e* 232 arising from the cleavage of the γ,γ-dimethylallyl group with hydrogen transfer. This type of cleavage has been noted frequently in the mass spectra of the linear furanocoumarins containing this ether group, such as imperatorin (III) (9, 10) and isoimperatorin (I) (9) or the closely related epoxides such as oxypeucedanin (IV) and prangenin (X) (9, 10). The peak at *m/e* 69 (13.8%) probably resulted from the loss of $CH_2{=}CH{-}\overset{+}{C}(CH_3)_2$ as suggested by Vul'fson *et al.* (9) and is characteristic for demonstrating the presence of a γ,γ-dimethylallyloxy group which also existed in imperatorin (III) and isoimperatorin (I) (9). The ion of *m/e* 231 (14.9%) arising from the M-69 cleavage or from the base peak by loss of H· is comparable to the one (XI or XII) obtained by losing a methyl radical from an aromatic methoxyl group of isopimpinellin (V) reported recently by Furuya *et al.* (11). Thus, the subsequent fragmentation of XI obtained from phellopterin, *i.e.*, the loss of a methyl radical from the methoxyl group followed by the subsequent loss of carbon monoxide is, in fact, the same as Furuya *et al.*

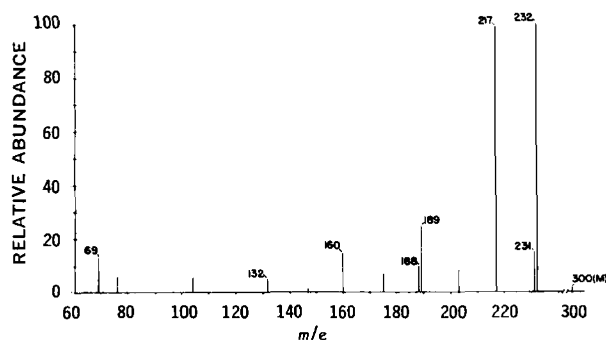
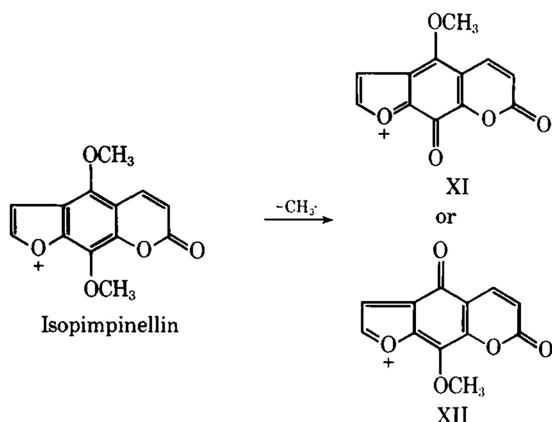


Figure 1—*Mass spectrum of phellopterin.*

(11) have proposed for one of the possible *m/e* 231 fragments (*i.e.*, XI) from isopimpinellin (see Scheme I).



Scheme I

The plausible mode of fragmentation, together with the abundance ratios of the most intense peaks of phellopterin, is summarized in Scheme II. [In Scheme II, M* indicates that the proposed transition is supported by a metastable ion. The instrument was operated at a source temperature 250° and an ionizing voltage 75 ev. The T.I.C. is 2.5×10^{-9} (temp. 150°).]

The mass spectrum of Compound VI (see Fig. 2) showed similar fragmentation to that of phellopterin and is shown in Scheme III. [For Scheme III, the spectrum of VI was kindly determined by the Department of Chemistry, College of Pharmacy, University of Illinois. The instrument was operated at a source temperature 200° and an ionizing voltage 70 ev. The sample inlet temperature was 60°. M* indicates that the proposed transition is supported by a metastable ion.]

Further acid cleavage of Compound VI resulted in a phenol (XIII), m.p.[3] 293–295°, which showed a hydroxy absorption band in the IR spectrum in mineral oil together with a bathochromic shift upon the addition of sodium hydroxide in the UV spectrum. Acetylation of XIII furnished the corresponding acetate (XIV), m.p.[4] 205–207°. The NMR spectrum of this compound is in full agreement with Structure XIV, revealing signals at 7.53 (3H, s, OCOCH5 (3H3), 5.7, s, OCH3), 3.65 (1H, d,

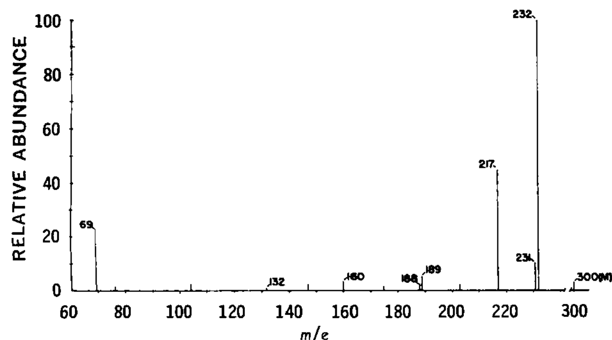
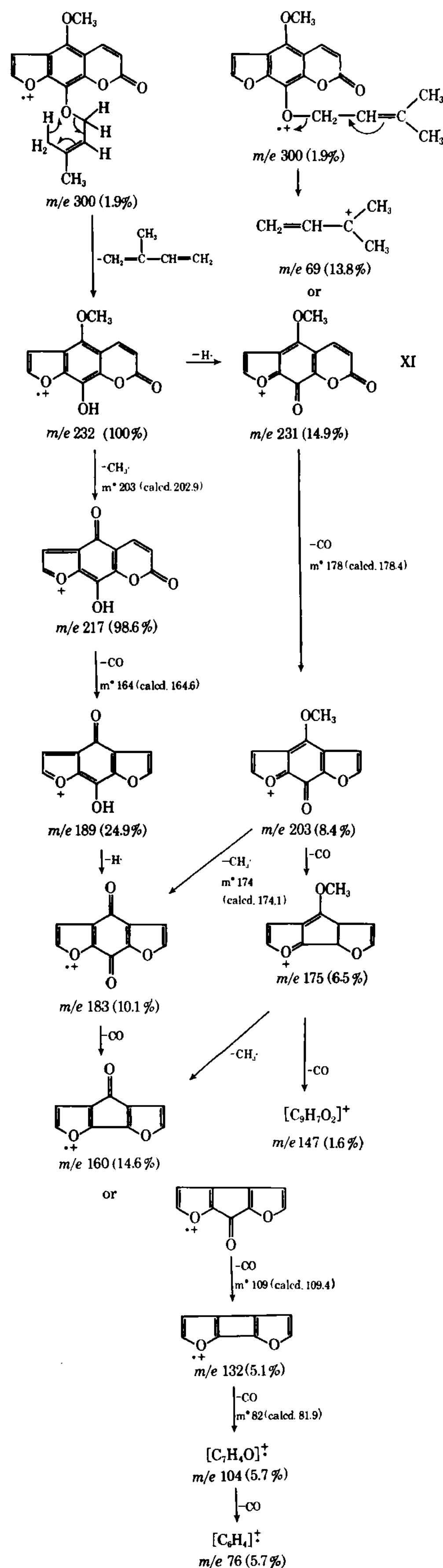
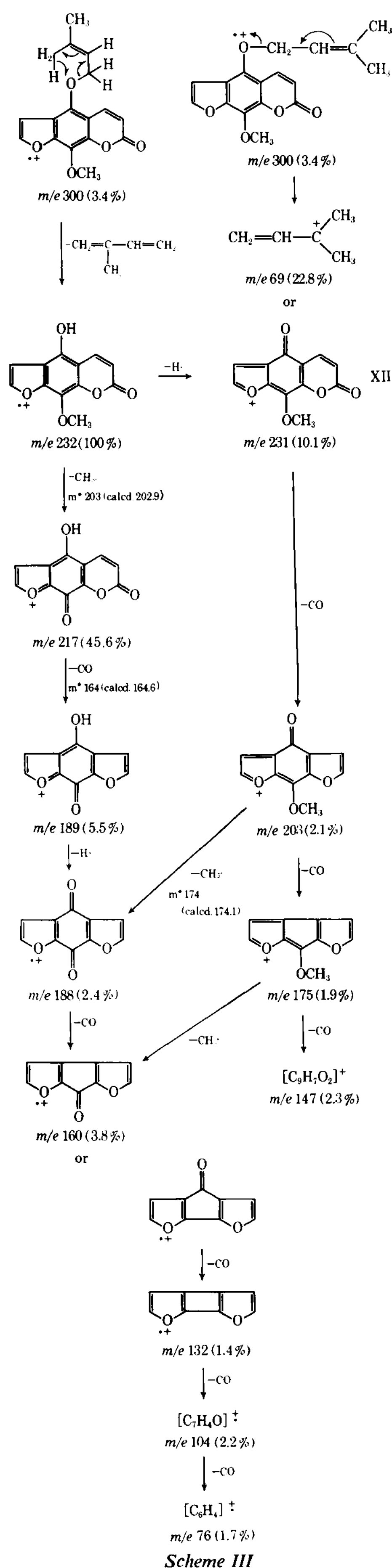


Figure 2—*Mass spectrum of Compound VI.*

[3] Lit. (12) reported m.p. about 260 or 270° with decomposition for a compound tentatively assigned this structure.
[4] This melting point is identical with that of a tentatively identified compound, 5-acetoxy-8-methoxy psoralen (XIV), reported by Stanley *et al.* (12). It was not possible to obtain an authentic sample but the data of Stanley *et al.* combined with the data in this paper conclusively identifies it as XIV.



Scheme II



Scheme III

C_3-H, $J_{3,4}$ = 10 c.p.s.), 2.23 (1H, d, C_4-H, $J_{3,4}$ = 10 c.p.s.), 3.35 (1H, d, C_4'-H, $J_{4',5'}$ = 2 c.p.s.), and 2.37 τ (1H, d, C_5'-H, $J_{4',5'}$ = 2 c.p.s.). Methylation of XIII yielded yellow needles, m.p. 145–146°. The identity of this compound with an authentic sample of 5,8-dimethoxypsoralen, *i.e.*, isopimpinellin (V) was established by direct TLC, IR spectroscopic comparison in chloroform, and mixed melting point determination. From the above degradative evidence, the structure of VI was proven to be 5-γ,γ-dimethylallyloxy-8-methoxy psoralen.[5]

The methanol extract showed a positive Molisch test and was worked up as described in the *Experimental* section. The extract finally provided sucrose in 0.77% yield and the remaining solids in 7.91% yield.

A preliminary paper chromatographic examination[6] of the nonsucrose solids revealed only one intense bright blue fluorescent spot under UV light. Purification of the solids by recrystallization provided a white compound, m.p. 215–217°, which after further recrystallization yielded colorless crystals, m.p. 219.5-221° and exhibited polymeric hydroxyl absorption in the IR spectrum in mineral oil. Acetylation of the above white compound with pyridine–acetic anhydride afforded a corresponding tetraacetyl glycoside (XV) which showed a lack of polymeric hydroxyl absorption in the IR spectrum, and its NMR spectrum indicated a tetraacetate peaking at 8.16 (3H, s), 8.02 (3H, s), 7.98 (3H, s), and 7.94 τ (3H, s). Acid hydrolysis of the foregoing white compound by refluxing with ethanolic sulfuric acid gave an aglycone and a sugar moiety. The sugar moiety was obtained as glucosazone which was identified by direct IR comparison with an authentic sample prepared from D-glucose. The aglycone showed m.p. 187–188°, and a molecular ion peak at *m/e* 246 in its mass spectrum; its NMR spectrum peaked at 8.77 (3H, s), and 8.63 (3H, s) (=C<CH_3 CH_3), 7.92 (1H, OH), 6.80 (2H, d, —CH_2, J = 10 cps.), 5.30 (1H, q, —CH—, J = 10 c.p.s.), 3.87 (1H, d, C_3-*H*, $J_{3,4}$ = 10 c.p.s.), 2.47 (1H, d, C_4-H, $J_{3,4}$ = 10 c.p.s.), 3.35 (1H, s, C_8-H) and 2.84 τ (1H, s, C_5-H). The identity of this compound with an authentic sample of nodakenetin (XVI) was established by direct comparison through mixed melting point and IR spectra.

Further acetylation and acid hydrolysis, respectively, of the mother liquor of the white compound by the same procedure described before, however, only yielded the same corresponding tetraacetyl glycoside (XV) and nodakenetin (XVI).

In order to further identify the sugar moiety, the above glycoside (m.p. 215–217°) was partially hydrolyzed by using a cation-exchange resin in the acid cycle. This indicated only the presence of a monosaccharide, namely glucose, as established by paper chromatog-

[5] Following the submission and acceptance of this manuscript for publication it has come to the authors' attention that cnidilin described by Komissarenko *et al.* [*Khim. Prir. Soedin.*, 2, 375(1966)] is undoubtedly identical to Compound VI. Accordingly, the terminology of the previous authors is accepted and the name cnidilin for VI is assigned. The present paper provides a substantial amount of additional data over that presented by the earlier authors.

[6] This examination is not described in the experimental section and, therefore, it may be of interest to note that it was carried out on Whatman paper No. 1 in pyridine–ethyl acetate–water = 2:5:7, upper layer; descending method.

raphy. The β-D-glucosidic linkage of the glucoside was demonstrated by enzymatic hydrolysis of the glucoside (m.p. 215–217°) with β-glucosidase in about 70% yield.

The evidence thus supported the structure XVII (*i.e.*, nodakenetin-β-D-glucoside) for the glycoside (m.p. 215–217°). XVII and its tetraacetate (XV) were subsequently compared with authentic samples of nodakenin and tetraacetyl nodakenin by direct comparison of mixed melting point and IR spectra to establish their identity beyond doubt.

EXPERIMENTAL[7]

Values of [α] were determined on a polarimeter.[8] UV spectra were determined on a recording spectrophotometer,[9] IR spectra on an infrared spectrophotometer,[10] and NMR spectra on a spectrometer[11] in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. s refers to singlet, d to doublet, t to triplet, and q to quartet. Mass spectra were determined on a mass spectrometer.[12] Silica gel was used for column chromatography[13] and for TLC.[14]

Material[15]—*Sphenosciadium capitellatum* (A. Gray) is a species belonging to the *Umbelliferae* family of plants. It is also known as "Ranger's Button" or "White Heads" and has the following pseudonyms: *Selinum eryngifolium* (Greene), *S. validum* (Congd.), *S. capitellatum* (S. Wats.), *Sphenosciadium c.* vars. *scabrum*, *e.* and *v.* (Jeps.) (13, 14). The present investigation was confined entirely to the roots of the plant which had been collected and identified in 1965 in Tulare County, Calif. The roots, which had been stored in a deep freeze refrigerator, were reduced to a powder suitable for extraction in a laboratory grinder (Jacobsen) designed to keep frictional heat at a minimum.

Aliphatic Naphtha Extract—The dried and ground root (700 g.) was extracted in a continuous extraction apparatus with aliphatic naphtha for 15 hr. until only a faint fluorescence was evident in the menstruum when spotted on filter paper and examined under UV light. The marc was dried and reserved for ether and methanol extraction (q.v.).

The aliphatic naphtha was evaporated *in vacuo* to give a dark-brown syrup (92 g.). This syrup was dissolved in 90% methanol, defatted with aliphatic naphtha, and evaporated to afford an orange viscous oil (50 g.) which was then chromatographed on silica gel (450 g., activated at 120° and impregnated with 10% of water). The column was successively eluted with benzene, benzene–chloroform, chloroform, chloroform–ethyl acetate, ethyl acetate, and ethyl acetate–methanol. Five hundred and fifty three 20-ml. fractions were collected and the composition of the fractions determined by examining thin-layer chromatograms under UV light. The first benzene eluate (Fractions 1–50) contained a mixture of two fluorescent spots in an orange-colored syrup (23 g.). The subsequent fractions (Fractions 51–95) (14.2 g.) appeared to be a mixture of the same fluorescent compounds.

A mixture of fluorescent substances was also obtained from the following fractions, *i.e.* benzene–chloroform (8:1) eluate [(Fractions 96–110) (0.6 g.)] and benzene–chloroform (3:1) eluate [Fractions 111–131 (1.3 g.), 132–163 (2.0 g.), and 164–193 (0.8 g.)]. Fractions 194–213 (0.4 g.) from benzene–chloroform (3:1) yielded a single spot having a greenish-blue fluorescence under UV light.

The following Fractions 214–263, 264–283, and 284–333 obtained from chloroform eluate, Fractions 334–403 from chloroform-ethyl acetate (3:1), Fractions 404–453 from chloroform–ethyl acetate (1:1), Fractions 454–503 from ethyl acetate, and Fractions 504–553 from ethyl acetate–methanol (9:1) all contained only traces of polar fluorescent substances and were not investigated further.

Isolation of Coumarins from the Aliphatic Naphtha Extract—The fractions containing fluorescent materials were worked up to yield the following.

Isoimperatorin (I)—The orange-colored syrup (23 g.) resulting from the removal of solvent from Fractions 1–50 was allowed to stand for 1 month at room temperature. The deposited crystals were removed by decantation and washed with aliphatic naphtha, followed with benzene. The colorless crystals (9.0 g.) obtained had m.p. about 92° and were recrystallized from acetone–aliphatic naphtha to give colorless needles with m.p. 104–107°. Two more recrystallizations from aliphatic naphtha afforded m.p. 106–108°. The melting point of this compound was undepressed on admixture with an authentic sample of isoimperatorin and their IR spectra were identical.

Phellopterin (II)—After the above decantation for the isolation of isoimperatorin, the syrup (13.3 g.) that remained was chromatographed on silica gel (75 g.), activated at 110° for 24 hr., and eluted with aliphatic naphtha–benzene (2:1), aliphatic naphtha–benzene (1:1.5), chloroform–benzene (1:1), and chloroform, respectively. The aliphatic naphtha–benzene (2:1) eluate (120 ml.) afforded 2.3 g. of isoimperatorin after one recrystallization from aliphatic naphtha. The aliphatic naphtha–benzene (1:1.5) eluate (900 ml.) yielded 5.5 g. of a mixture of isoimperatorin and a small amount of other fluorescent compounds which showed identical TLC behavior with that of the subsequent benzene–chloroform (1:1) eluate. The chloroform eluate gave only traces of a mixture of fluorescent compounds which were virtually identical on TLC compared with that of Fractions 96–110.

The benzene–chloroform (1:1) eluate (1:7.1) provided an orange-yellow oil (2.7 g.) which upon addition of absolute ether furnished a crystalline residue. One recrystallization of this residue from acetone–aliphatic naphtha gave pale yellow crystals (2.0 g.), m.p 75–88°, which upon being recrystallized three times from aliphatic naphtha–ethyl acetate (3:1) yielded fine colorless prisms (294 mg.), m.p. 101–102°. A mixed melting point with authentic phellopterin[16] showed no depression and the IR spectra were identical.

Compound VI—The mother liquor, after removal of the preceding crystalline phellopterin, was evaporated under reduced pressure to yield a residue (890 mg.). This residue was rechromatographed on silica gel (13 × 2.1 cm.) activated at 110° for 24 hr. and the aliphatic naphtha–ethyl acetate (3:1) eluate was evaporated to give a crystalline residue which was recrystallized from acetone–aliphatic naphtha and then from absolute ethanol to provide fine slender yellow needles (87 mg.), m.p. 109–117°. Two more recrystallizations of this compound from anhydrous ether yielded fine colorless needles, m.p. 115–115.5°. IR $\nu_{max.}^{mineral\ oil}$ cm.$^{-1}$: 3120, 3078, 3025 (ν CH of furans) (15), 1715 (α-pyrone C=O), 1620 (shoulder), 1600 (shoulder), 1587, 1540 (aromatic C=C), and 880 (sharp and medium, characteristic peak of furans) (15, 16). UV $\lambda_{max.}^{EtOH}$ mμ (log ε): 223 (4.36), 242 (4.11), 249 (4.12), 270 (4.20), and 313 (4.02).

Anal.—Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 68.29; H, 5.54.

Imperatorin (III)—The orange-colored oily substance (0.6 g.) obtained from Fractions 96–110 crystallized upon addition of anhydrous ether. The yellow crystals were collected and twice recrystallized from ethyl acetate-anhydrous ether to give colorless plates (110 mg.), m.p. 98–100°. It failed to depress the melting point of an authentic sample of imperatorin on admixture and the IR spectra were identical.

Isopimpinellin (V)—The yellow oily substance (1.3 g.) resulting from Fractions 111–131 was treated with a small amount of acetone and allowed to stand for some time. The yellow crystals deposited were filtered (90 mg.) and recrystallized from acetone to give yellow needles (23 mg.), m.p. 146–147°. The identity of this compound with an authentic sample of isopimpinellin[17] was established by

[7] Unless otherwise specified, melting points were determined in capillary tubes in a Thomas-Hoover m.p. apparatus, checked for accuracy against a set of standard samples and are uncorrected. Microanalyses were determined by the Microanalytical Laboratory, School of Chemistry, University of Minnesota.
[8] Perkin-Elmer 141.
[9] Cary model 14.
[10] Perkin-Elmer 237B grating.
[11] Varian Associates A-60.
[12] Hitachi Perkin-Elmer RMU-6D.
[13] Baker Analyst No. 3405.
[14] Silica Gel G, activated at 120°, Brinkmann Instruments, Inc., Great Neck, N. Y.
[15] Collected and initially identified by Dr. T. G. Call, California State Polytechnic College, San Luis Obispo, Calif. with confirmation of the identification by Dr. G. B. Ownbey, Department of Botany, University of Minnesota, Minneapolis, Minn. A voucher specimen has been placed in the herbarium of the Botany Department, University of Minnesota.

[16] Kindly provided by Dr. A. Chatterjee, University of Calcutta, Calcutta, India, and Dr. K. Hata, Kyoto University, Kyoto, Japan.
[17] Kindly provided by Dr. H. Abu-Shady, Cairo University, Cairo, Egypt.

TLC, IR spectroscopic comparison in chloroform and in mineral oil, and mixed melting-point determination.

Oxypeucedanin (IV)—The solids obtained from Fractions 132–163 after evaporation of solvent weighed 2.0 g. and were recrystallized from acetone to yield fine colorless crystals (270 mg.), m.p. 139–141°. The identity of this compound with an authentic sample of oxypeucedanin[18] was confirmed by mixed melting point, TLC, and superimposable IR spectra.

Acid Cleavage of Compound VI—A solution of VI (300 mg.) in glacial acetic acid (3 ml.) was treated with 2 drops of concentrated sulfuric acid. The yellow precipitate which formed within 1 min. was filtered, and was washed well with cold glacial acetic acid followed with cold water. Two recrystallizations from 95% ethanol afforded small yellow crystals (XIII) (195 mg.), m.p. 293–295°. IR $\nu_{max.}^{mineral\ oil}$ cm.$^{-1}$: 3325 (OH). UV $\lambda_{max.}^{EtOH}$ mμ (log ε): 224 (4.22), 241 (3.94, shoulder), 248 (3.87, shoulder), 268 (4.00, shoulder), 275 (4.10), 297 (3.80, shoulder), 316 (3.87), and 328 (3.81, shoulder), and upon addition of sodium hydroxide, it exhibited $\lambda_{max.}^{EtOH}$ mμ (log ε): 232 (4.20), 292 (4.17),[19] 324 (3.73), 332 (3.70, shoulder), and 337 (3.67, shoulder).

Acetylation of XIII—A mixture of the foregoing phenol (XIII) (40 mg.), fused sodium acetate (40 mg.), and acetic anhydride (2 ml.) was heated on the steam bath, with occasional shaking, for 4 hr. until TLC showed that the slower-moving fluorescent phenol spot had disappeared completely in favor of a much faster-moving fluorescent ester spot. The mixture was poured, with stirring, into ice water (25 ml.) and the resulting precipitate was filtered, washed thoroughly with water, and recrystallized from 95% ethanol, white needles (XIV) (30 mg.), m.p. 205–207°. IR $\nu_{max.}^{mineral\ oil}$ cm.$^{-1}$: 1760 (vinyl ester C=O), 1200 (strong, antisymmetric stretching of =C—O—C=), 1036 (strong, symmetric stretching of =C—O—C=), and absence of hydroxyl absorption. UV $\lambda_{max.}^{EtOH}$ mμ (log ε): 219 (4.30), 243 (4.19, shoulder), 249 (4.25), 263 (4.18), and 303 (4.02). Lit. (12) reports m.p. 205–207°.

Methylation of XIII—A mixture of XIII (50 mg.), anhydrous potassium carbonate (50 mg.), anhydrous acetone (5 ml.), and methyl iodide (1 ml.) was refluxed on a steam bath for 2 hr. The solvent was evaporated *in vacuo* and the residue was column-chromatographed on silica gel (1.7 × 7 cm.). Elution with benzene-chloroform (1:1), after evaporation, yielded a yellowish crystalline residue which was recrystallized from 95% ethanol to yield yellow needles, m.p. 145–146°. A mixed melting point with authentic isopimpinellin showed no depression and the IR spectra in chloroform were identical.

Methanol Extract—The marc from the ether extraction was dried in air and extracted with methanol for 5 days until only a faint fluorescence was evident in the menstruum when spotted on filter paper and examined under UV light. The hot extract was filtered and allowed to concentrate spontaneously at room temperature. After 1 month, yellow solids began to deposit as the volume decreased. The yellow solids were filtered, rinsed well with absolute methanol, and dried to give a pale yellow solid (22.4 g.), m.p. 205–210°, which was recrystallized from absolute methanol to form a white solid, m.p. 215–217°. Two recrystallizations from absolute methanol afforded colorless crystals, m.p. 219.5–221° [lit. (17) reported nodakenin, m.p. 215°]. IR $\nu_{max.}^{mineral\ oil}$ cm.$^{-1}$: 3470, 3320–3220 (polymeric OH), 1720 (α-pyrone C=O), 1621, 1568, 1480 (aromatic C=C), 1160–1021 (various C—O—C stretching). The melting point of this compound was not depressed by admixture with nodakenin[20] (XVII) and their IR spectra were also identical.

Acetylation of Nodakenin—The above white solid (m.p. 215–217°) (200 mg.) was treated with anhydrous pyridine (2 ml.) and acetic anhydride (1 ml.) and the mixture, after leaving at room temperature for 24 hr., was treated with water (15 ml.). The resulting crystals were filtered, washed well with water, and dried to furnish white tufts (231 mg.). Two recrystallizations from absolute methanol yielded fine colorless tufts, m.p. 193–194°. Lit. (18) reported m.p. of tetraacetylnodakenin (XVI) 195–196°. NMR (τ): 8.71 (3H, s), 8.63 (3H, s) (=C(CH$_3$)(CH$_3$)), 8.16 (3H, s), 8.02 (3H, s), 7.98 (3H, s), 7.94 (3H, s) (OCOC$\underline{H}_3$), 3.81 (1H, d, C$_3$-$\underline{H}$, $J_{3,4}$ = 10 c.p.s.), 2.41 (1H, d, C$_4$-$\underline{H}$, $J_{3,4}$ = 10 c.p.s.), 3.32 (1H, s, C$_8$-$\underline{H}$), and 2.80 (1H, s, C$_5$-$\underline{H}$). The IR spectrum of this compound was identical with that of tetraacetylnodakenin.[20]

Anal.—Calcd. for $C_{28}H_{32}O_{13}$: C, 58.31; H, 5.60. Found: C, 58.50; H, 5.74.

Acid Hydrolysis of Nodakenin—The foregoing white solid (m.p. 215–217°) (400 mg.) was refluxed with 10% sulfuric acid in 30% ethanol (25 ml.) for 30 hr. After being similarly refluxed with a further portion of 10% sulfuric acid in 30% ethanol (5 ml.) for 24 hr., the mixture was diluted with water (30 ml.) and extracted four times with chloroform (60 ml.) until the aqueous phase showed only a faint fluorescence when spotted on filter paper and examined under UV light.

The chloroform phase was washed with water, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to yield a crystalline residue (180 mg.). Recrystallization from acetone gave colorless scales, m.p. 187–188°. UV $\lambda_{max.}^{EtOH}$ mμ (log ε): 224 (3.99), 248 (3.59), 258 (3.50), 298 (3.69, shoulder), and 336 (4.18). IR $\nu_{max.}^{mineral\ oil}$ cm.$^{-1}$: 3415 (OH), 1150 (C—O of *tert* alcohol), 1700 (conj. α-pyrone C=O), and 1627, 1563 (aromatic C=C). The melting point was not depressed on admixture with an authentic sample of nodakenetin[20] (XVI) and the IR spectra were identical.

The acidic aqueous phase was made just neutral with a saturated solution of barium hydroxide. The precipitated barium sulfate was filtered off and the filtrate was concentrated *in vacuo* to furnish a white residue (240 mg.). A solution of this residue (130 mg.) in water (2 ml.) was treated with fused sodium acetate (100 mg.), phenylhydrazine (6 drops), and glacial acetic acid (6 drops), and the mixture was heated in a boiling-water bath for 30 min. The resulting yellowish crystals were filtered, washed with a small amount of water containing glacial acetic acid, followed with water, and finally dried. Recrystallization from aqueous methanol afforded glucosazone (90 mg.) as yellowish-brown crystals, m.p. 205–206°. Lit. (19) reported m.p. 205°. The melting point was not depressed on admixture with a sample of glucosazone prepared from D-glucose. The IR spectra of the two compounds were also identical.

Partial Hydrolysis of Nodakenin—To a solution of the foregoing white compound (m.p. 215–217°) (20 mg.) in water was added 40 mg. of cation-exchange resin in the acid cycle[21] in a small test tube and the mixture was heated in a boiling water bath for 2 hr. The hydrolysate was spotted on standard length Whatman paper No. 1, together with authentic samples of glucose and maltose, and developed in pyridine–ethyl acetate–water (2:5:7, upper layer), by the descending method for about 16 hr. After drying, the paper was then developed with silver nitrate in acetone in the usual manner (20). Only a single spot with an R_f value identical to that of D-glucose was observed.

Enzymatic Hydrolysis of Nodakenin—The above white compound (m.p. 215–217°) (200 mg.) was dissolved in water (20 ml.) with the aid of heat and, after cooling, 100 mg. of β-glucosidase[22] (800 units/mg.) was added. The mixture was slowly stirred for 3 hr. at room temperature. After being similarly treated with another 100 mg. of β-glucosidase followed by an additional 30 hr. of stirring, the product was extracted with chloroform (4 × 15 ml.). The chloroform extract was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to yield a white crystalline residue (100 mg.). One recrystallization from acetone provided fine, bright, colorless, slender needles, m.p. 186–187°. This compound showed no depression in melting point on admixture with the foregoing nodakenetin isolated from the acid hydrolysis of nodakenin and the IR spectra were identical.

Isolation of Sucrose—The mother liquor, after the removal of the solids (*i.e.*, nodakenin, m.p. 205–210°), was further allowed to stand at room temperature for 1 month until it became very concentrated (volume, about 30 ml.). The solids that separated were filtered, washed with methanol, and dried to provide a pale yellow substance (33 g.), m.p. 205–210°. The mother liquor of this compound was again allowed to stand at room temperature for a few weeks. The colorless cubes that deposited were collected, rinsed well with absolute methanol, and dried, m.p. 185–187°, and weighed (5.4 g.). The IR spectrum of this compound showed $\nu_{max.}^{mineral\ oil}$ cm.$^{-1}$: 3525, about 3200–3400 (broad, strong) (dimeric and polymeric OH), 1030–1150 (various C—O—C stretching), and the absence

[18] Kindly supplied by Dr. K. Hata, Kyoto University, Kyoto, Japan.
[19] This major absorption peak was not reported by lit. (12).
[20] Kindly supplied by Dr. M. Pailer, University of Vienna, Vienna, Austria.
[21] Marketed as Amberlite IR-120 by Rohm and Haas Co., Philadelphia, Pa. and converted to the acid cycle with 10% sulfuric acid.
[22] Product of General Biochemicals, Laboratory Park, Chagrin Falls, Ohio.

of C=O and aromatic C=C absorption bands and was identical with that of an authentic sample of sucrose.

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Coumarins X: Spectral Studies on Some Linear Furanocoumarins

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Abstract ☐ Observations on the IR, UV, and NMR spectral data of some substituted psoralen-type linear furanocoumarins are presented as an aid to their differentiation.

Keyphrases ☐ Furanocoumarins, linear—spectral studies ☐ NMR spectroscopy—structure, identification ☐ UV spectrophotometry—structure, identification ☐ IR spectrophotometry—structure, identification

During an investigation of the coumarin content of *Sphenosciadium capitellatum* (A. Gray) a series of biogenetically closely-related methyl or isoprenyl ether-substituted psoralen-type (I) linear furanocoumarins has been obtained (1). The present paper reports the direct comparison of their spectral properties together with those of certain other closely related compounds (VIII, IX, and X) in order to permit their ready differentiation. The results are summarized in tabular form and are discussed as to the significant differences.

DISCUSSION

NMR Spectral Comparison—The results of NMR studies are summarized in Table I. The chemical shifts and coupling constants of C_3-H, C_4-H, C_4'-H, and C_5'-H of isoimperatorin (II), isopimpinellin (VII), and imperatorin (VI) have been reported previously by Sheinker *et al.* (2) and Abu-Mustafa *et al.* (3), respectively. However, the former measured II, VI, and VII in CCl_4 and reported $J_{4',5'}$ = 2 c.p.s., whereas the latter measurements were in $CDCl_3$ and $J_{4',5'}$

Table I—NMR Comparison of Furanocoumarins

		Chemical Shifts (τ)										Coupling Constants (c.p.s.)		
		—OC$\underline{H}_3$	=C(C$\underline{H}_3$)$_2$	—OC$\underline{H}_2$	—C$\underline{H}$=	3-$\underline{H}$	4-$\underline{H}$	5-$\underline{H}$	8-$\underline{H}$	4'-$\underline{H}$	5'-$\underline{H}$	J-C$\underline{H}_2$,—C$\underline{H}$=	$J_{3,4}$	$J_{4',5'}$
No.	Compd.	s[a]	s	d[b]	t[c]	d	d	s	s	d	d			
II	Isoimperatorin		8.30 8.20	5.14	4.50	3.82	1.92		2.95	3.09	2.47	7	10	2
III	Oxypeucedanin		8.66 8.58			3.71	1.80		2.84	3.03	2.38		10	2
IV	Cnidilin	5.87	8.33 8.22	5.21	4.44	3.74	1.85			3.07	2.38	7	10	2
V	Phellopterin	5.87	8.32	5.20	4.43	3.80	1.94			3.05	2.41	7	10	2
VI	Imperatorin		8.29	5.05	4.44	3.71	2.27	2.71		3.23	2.33	7	10	2
VII	Isopimpinellin	5.86				3.79	1.97			3.07	2.44		10	2

[a] s = singlet. [b] d = doublet. [c] t = triplet.