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STUDIES ON CONSTITUENTS OF *THAMNOSMA MONTANA* TORR. AND FREM.

THE STRUCTURE OF THAMNOSMIN, A NOVEL COUMARIN EPOXIDE*

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Abstract—An intensive investigation on the natural products found in *Thamnosma montana* Torr. and Frem. is described and the identification of fifteen constituents is discussed. Of those compounds previously shown to occur in the plant, a number of coumarins were recognized for the first time. Two of these constituents were novel, alloimperatorin methyl ether epoxide (**11**) and thamnosmin (**19**), the latter possessing a unique system not previously encountered in nature.

THAMNOSMA MONTANA Torr. and Frem. (Rutaceae), known as the turpentine broom, has proven to be a prolific source of benzenoid natural products,[1,2] and in particular, of some novel coumarins.[3–5] Bennett and Bonner[1] were able to isolate and identify the coumarins byakangelicin (**1**) and isopimpinellin (**2**) while Dreyer[2] identified as constituents; β-sitosterol (**3**), N-methylacridone (**4**), γ-fagarine (**5**), skimmianine (**6**), and the coumarins alloimperatorin methyl ether (**7**) and 5(2′,3′-dihydroxy-3′-methylbutyl)-8-methoxypsoralen (**8**) (the 2′,3′-diol of alloimperatorin methyl ether). Investigations in our laboratory[3,4] revealed a ninth component as the novel dimeric coumarin, thamnosin (**9**).

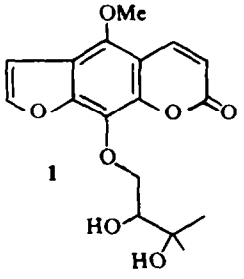
During preliminary experiments in the study of the biosynthesis of coumarins in *Thamnosma montana* it became evident that additional unreported phenylpropanoid constituents were present in isolable quantities in the plant. To more clearly define the possibilities for biosynthetic investigations in this species, a detailed investigation of these unknown constituents was undertaken.

For our purposes, the plants were divided into two portions; (a) roots and root crown, and (b) shoots, stems and leaves. In each case the plant material was air dried, ground to a coarse powder and extracted with acetone. The crude, oily extracts thus obtained were treated with chloroform, and the chloroform soluble portions subjected to column and preparative layer chromatography as required to effect purification of the desired components.

Processing of the shoots portion in this manner allowed isolation of four crystalline compounds. Two of these were readily identified as isopimpinellin (**2**) and alloimperatorin methyl ether (**7**) by comparison with authentic samples.† The remaining two components did not correspond to any of the known constituents previously isolated. The least polar component was readily identified as umbelliprenin (**10**)[6] by

* Financial support from the National Research Council of Canada is gratefully acknowledged.

† Our gratitude is expressed to Dr. D. L. Dreyer for providing us with this sample.



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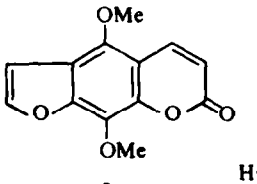

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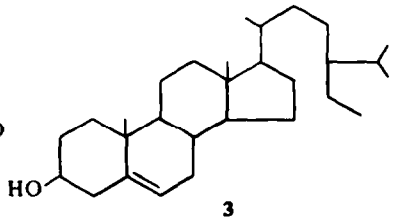

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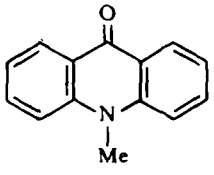

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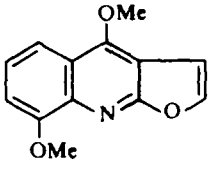

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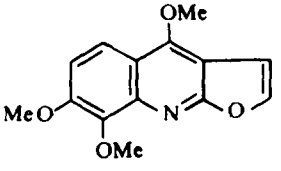

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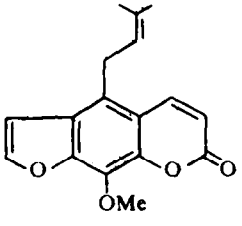

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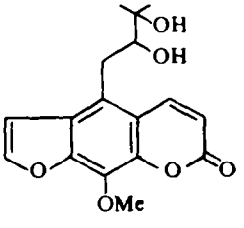

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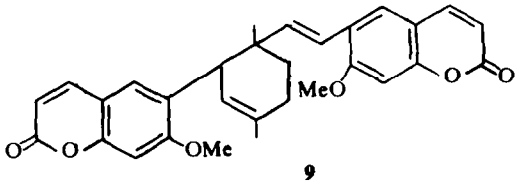

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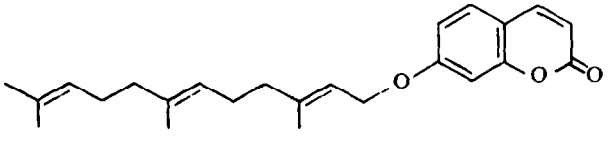

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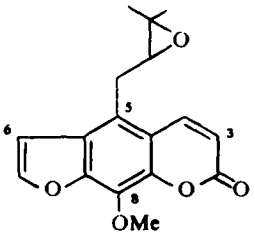

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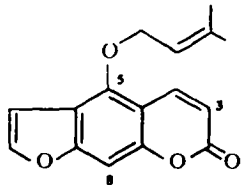

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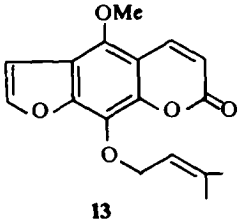

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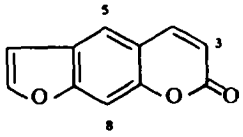

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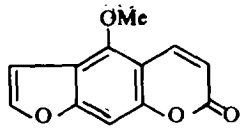

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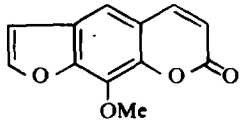

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appropriate comparison (IR, UV, mass and NMR spectra and mixed m.p.) with an authentic sample synthesized in our laboratory.[7]

The second and more polar component, a major constituent of the shoots, was, after careful scrutiny of the spectral data, assigned structure **11**, the 2',3'-epoxide of alloimperatorin methyl ether. Analytical and high resolution mass spectrometric data were in accord with the molecular formula $C_{17}H_{16}O_5$, and the UV spectrum was superimposable on that of alloimperatorin methyl ether thereby indicating a similar 8-oxygenated psoralen system.

The NMR spectrum of this compound contained signals in the aromatic region in accord with a 5,8-disubstituted furanocoumarin. Thus doublets at τ 1·82 (J 10 Hz, H(4)) and τ 3·64 (J 10 Hz, H(3), and doublets at τ 2·33 (J 2 Hz H(7)) and at τ 3·11 (J 2 Hz, H(8)) were the only signals in the aromatic region. A three proton singlet at τ 5·78 was readily assigned to an aromatic methoxyl group, necessarily in the 8-position. The remainder of the signals could be assigned to protons on a side chain attached in the 5-position. A three proton multiplet (J_{AB} 14·5 Hz, J_{AB} 14·5 Hz, J_{AC} 3 Hz, J_{BC} 7·5 Hz) in the region, τ 6·5–7·2, and two, three proton singlets at τ 8·51 and 8·70 completed the spectrum thereby accounting for a total of nine protons in the side chain. Considering the molecular formula and the requirement that **11** is an 8-methoxyfuranocoumarin, it follows that the side chain must contain the fragment C_5H_9O and one degree of unsaturation. The NMR evidence was therefore consistent with **11**, the epoxide of alloimperatorin methyl ether.

The mass spectrum was also in agreement with the proposed structure. The molecular ion at *m/e* 300 was accompanied by peaks at *m/e* 285 (M-Me), 271 (M-CHO), 257 (M-C_3H_7) and the base peak at *m/e* 229. This latter peak has been reported as the base peak in similarly substituted coumarins where oxygen functions replace the double bond in the side chain.[8] Its appearance is rationalized in terms of cleavage β to the aromatic ring and ring expansion of the resultant ion to form a tropylium-type ion (**22**). (Fig 1).

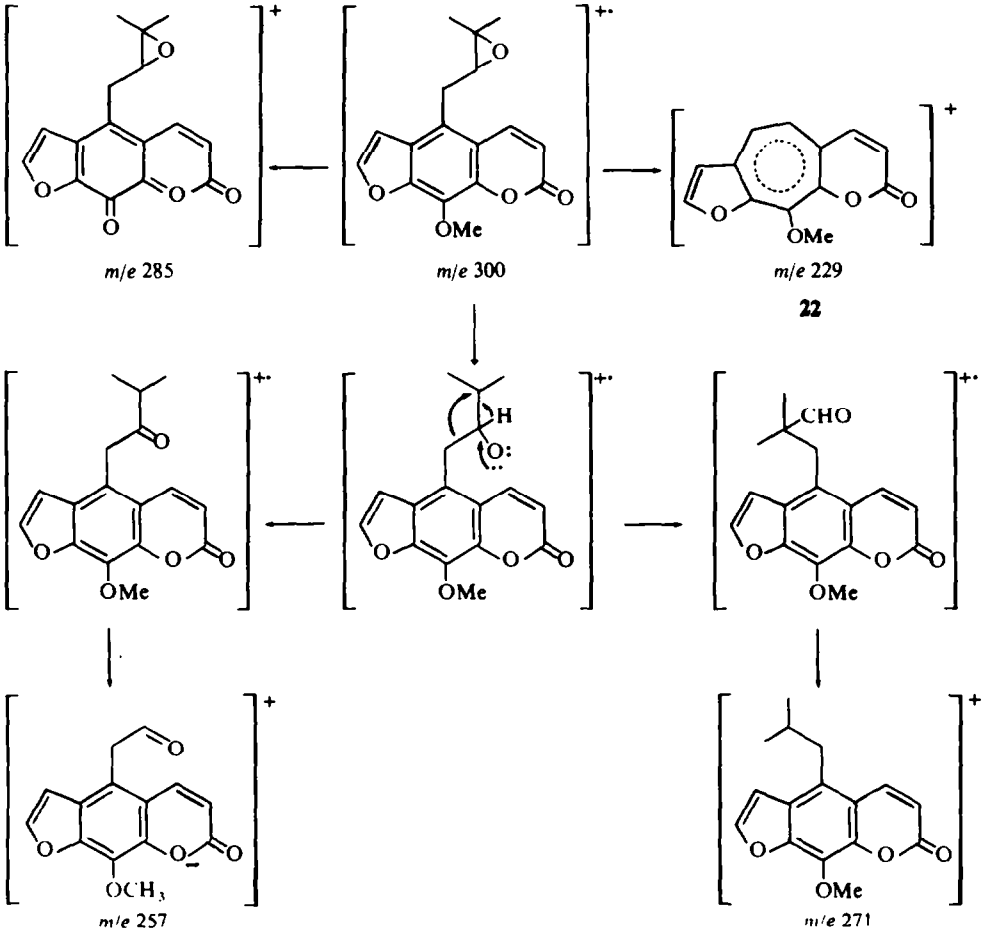


FIG 1. Proposed fragmentation of alloimperatorin methyl ether epoxide (**11**) in the mass spectrometer.

The fragmentations depicted in Fig 1 are fully consistent with rearrangements and fragmentations proposed by Djerassi *et al.*[9] for aliphatic epoxides.

The final proof of structure was obtained when alloimperatorin methyl ether was converted in good yield to epoxide **11** by *m*-chloroperbenzoic acid.[2] The synthetic material was identical in all respects with the natural compound. This is the first reported isolation of **11** from a natural source.

The root portion of the plant was processed in similar manner to that described

for the shoots. *Via* column and preparative layer chromatography several components could be isolated. Of the known components of *T. montana*; β-sitosterol (**3**), alloimperatorin methyl ether (**7**), γ-fagarine (**5**), N-methylacridone (**4**), thamnosin (**9**) and skimmianine (**6**) were readily isolated and identified. No evidence for the presence of diol **8** or byakangelicin (**1**) was noted in this extract. In addition to the previously reported components, six other crystalline compounds were isolated which did not correspond to any of the known constituents. Of these five proved to be known coumarins. The least polar component (also present in the shoots extract in small quantities), was identified as isoimperatorin (**12**). The molecular formula was shown to be $C_{16}H_{14}O_4$ and the UV spectrum was in keeping with a 5-oxygenated furanocoumarin.[11] The NMR spectrum contained signals in the aromatic region for five protons with the multiplet assigned to H (8) revealing small coupling constants consistent with long range coupling of this proton with H(4) (*J* 0·5 Hz) and H6 (1·0 Hz). This observation allowed the placement of this aromatic proton at the 8- and not the 5-position.[11] The mass spectrum of the compound was completely as expected. Thus the parent ion at *m/e* 270 was weak and except for the base peak at *m/e* 69 (representing the isoprenyl fragment) the remainder of the spectrum was essentially identical to that of bergaptol. Although an authentic sample of isoimperatorin was not readily available, it is felt that the spectroscopic and physical data (including the coincidence of the m.p. with that reported[12]) provides conclusive proof of structure.

Four more polar components isolated from the roots extract (but also evident in the shoots extract) were identified as phellopterin (**13**), psoralen (**14**), bergapten (**15**) and xanthotoxin (**16**) by appropriate spectral comparisons with authentic samples.[6]

Finally a component of intermediate polarity was isolated. This compound has been named thamnosmin in view of its close biogenetic relationship to the dimeric coumarin thamnosin (**9**), and on the basis of the following data has been assigned the novel structure **19**.

Elemental analysis and high resolution mass spectrometry of this substance established the molecular formula as $C_{15}H_{14}O_4$. The presence of the 7-alkoxy-

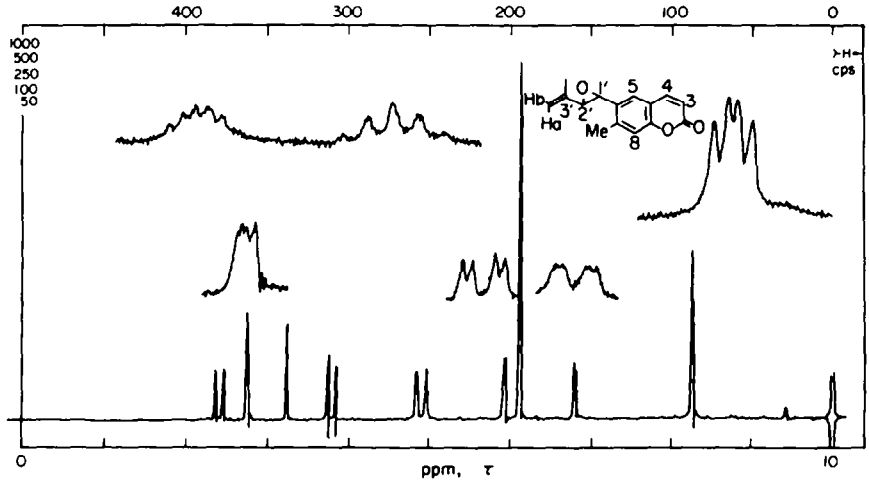
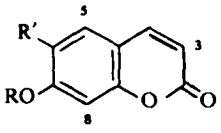


FIG 2. NMR spectrum of thamnosmin (**19**).

coumarin chromophore was indicated by the UV spectrum (λ_{max}^{MeOH} 227·5, 253 (sh), 297 (sh) and 327 nm) which was essentially identical with the UV spectrum of suberosin[13] while the IR spectrum contained the characteristic coumarin absorptions at 1722, 1610 and 1552 cm^{-1}. In addition, absorptions at 3075 and 903 cm^{-1} suggested the presence of a terminal methylene group.

The NMR spectrum (Fig. 2) again proved very instructive. The aromatic region of the spectrum contained the characteristic coumarin doublets at τ 2·42 (J 9·4 Hz, H(4)) and τ 3·77 (J 9·4 Hz, H(3)). Also in the aromatic region a broadened singlet at 2·77 and a sharp singlet at τ 3·25 were readily assigned to H(5) and H(8) respectively. In conjunction with the UV and IR evidence, these data allowed the proposal that this novel compound possesses a 6-substituted-7-alkoxycoumarin system as shown in **17**.



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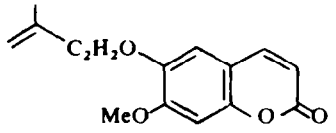
The presence of the coumarin doublets rules out substitution in either the 3- or the 4-position and the lack of significant splitting of the other two aromatic proton signals requires a *para* and thus 5,8-disposition of these two protons. A sharp three proton singlet at τ 6·13 was assigned to an aromatic OMe group which is of necessity placed in the 7-position. It follows that the remaining NMR signals must be assigned to protons involved with a side chain attached to the 6-position of the coumarin system.

Thus one proton multiplets at τ 4·85 and 4·97 were assigned as olefinic proton signals and a three proton multiplet at τ 8·25 was tentatively assigned to a vinyl Me group. A pair of apparent doublets at τ 5·93 (J 2 Hz) and 6·80 (J 2 Hz) completed the spectrum.

Considering the molecular formula of the unknown, it is evident that the side chain in the 6-position can be represented by the partial formula C_5H_7O. Also, the molecular formula demands nine degrees of unsaturation in the molecule of which seven can be assigned to the coumarin system, thereby leaving two sites of unsaturation in the side chain. With this in mind high resolution NMR spectra were obtained and spin decoupling studies were carried out.

Under high resolution (Fig. 2) the methyl multiplet at τ 8·25 was resolved as a doublet of doublets (J 1·5 and 0·9 Hz). The olefinic proton multiplet at τ 4·85 remained complex with at least seven broad lines being apparent (half-height width of this peak was 4 Hz). The higher field olefinic multiplet was resolved as a symmetrical quintet (J 1·5 Hz). Irradiation at the frequency of the methyl signal caused the collapse of the olefinic quintet to a doublet (J 1·5 Hz) while the lower field olefinic multiplet sharpened to an apparent doublet of doublets. It was thus evident that the side chain double bond was disubstituted with one of the substituents being a methyl group. The olefinic protons are coupled to each other with a coupling constant of 1·5 Hz and therefore must be geminal.[14a] Jackman[14b] has noted that spin–spin couplings across propene double bonds generally follow the relationship J *cis* > J *trans* by 0·3 to 0·6 Hz. Thus the olefinic proton absorbing at τ 4·97 reveals a coupling to the

methyl group of 1·5 Hz. On this basis a further partial structure of the unknown could be assigned as shown in **18**.



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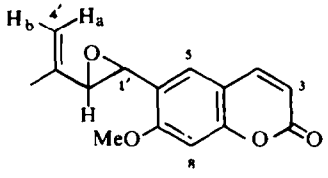
The small additional splitting evident in the τ 4·85 olefinic multiplet would appear to be due to small coupling with a *trans* allylic proton.

The residual fragment, C_2H_2O, containing one degree of unsaturation which still remains to be assigned, must be placed between the isopropylidene group and the aromatic ring. Only four basic arrangements of these atoms need be considered; an enol, an α-methylene ketone, an α-methyne aldehyde, or an epoxide. The enol possibility can be quickly eliminated as there are no exchangeable proton signals in the NMR spectrum. Also, an enolic group would provide further conjugation with the coumarin system and thus change the UV spectrum significantly from that observed. The aldehyde function can be eliminated as there are no characteristic low field signals in the NMR spectrum. A ketonic carbonyl adjacent to the aromatic ring would affect the UV spectrum and therefore can be eliminated from serious consideration. The remaining possibilities are portrayed in structures **19**, **20** and **21**.



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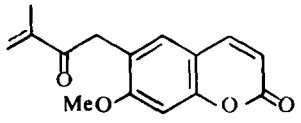

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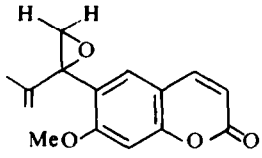

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Structure **20** is not entirely consistent with the NMR spectrum since the benzylic methylene signal would be expected to occur as a low field singlet. On the other hand structures **19** or **21** could fit the data well, although the former would be preferred on biogenetic grounds.

Further examination of the NMR spectrum of the unknown under high resolution and spin decoupling experiments allowed the assignment of structure **19** to the new natural product. The apparent doublets at τ 5·93 and 6·80 were assigned to the epoxide protons. Furthermore, high resolution revealed the τ 5·93 signal to be a doublet of doublets (J 2·0 Hz and 0·65 Hz) while the higher field doublet was somewhat broadened (J 2·0 Hz). Intuitively the additional splitting in the τ 5·93 signal would suggest that this signal is due to the proton which is involved in allylic coupling with H(4'a). On the other hand one would also expect the benzylic proton to absorb at lower field than the allylic epoxide proton. This paradox was resolved by double irradiation experiments. Coupling between the epoxide protons was confirmed since when the signal at τ 5·93 was irradiated the doublet at τ 6·80 collapsed to a singlet. When the olefinic proton H(4'a) signal at τ 4·85 was irradiated, the secondary splitting in the τ 5·93 signal remained unchanged, while the broadened doublet at τ 6·80 sharpened

noticeably. Examination of the aromatic region of the spectrum under high resolution shed some light on the nature of the observed couplings. The singlet at τ 2·77 assigned to H(5) was observed to be quite broad and when this signal was irradiated the doublet of doublets at τ 5·93 collapsed to a doublet (J 2·0 Hz). Thus this splitting (0·65 Hz) was due to long range coupling between H(5) and H(1'). Such benzylic coupling is not unknown and Jackman and Sternhell state that the coupling of a benzylic proton to the *ortho* proton is generally in the range 0·6–0·9 Hz with coupling to the *meta* proton being considerably smaller.[14c] The coupling constant of 2·0 Hz observed for the epoxide protons is indicative of a *trans* relationship between these protons.[15] On this basis it was felt that thamnosmin is best represented by structure **19**.

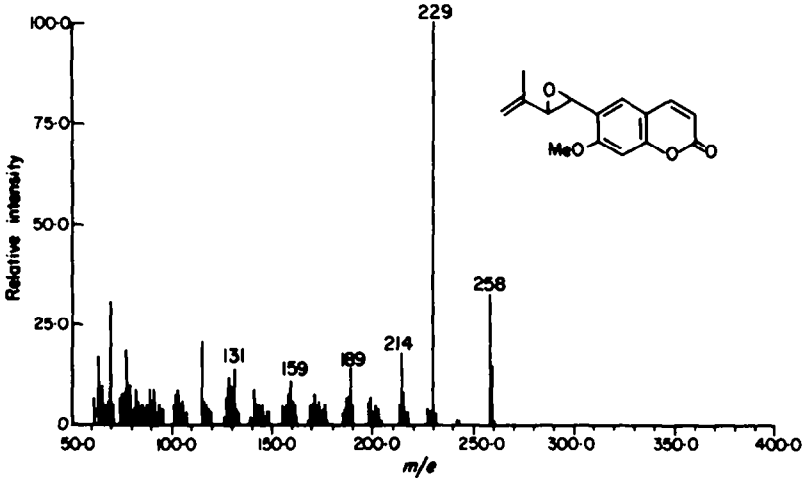
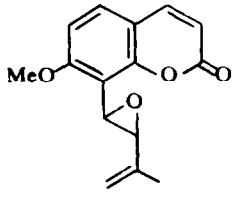


FIG 3. Mass spectrum of thamnosmin (**19**).

The mass spectrum (Fig 3) was in full agreement with the proposed structure. In addition to the molecular ion peak at *m/e* 258, the base peak at *m/e* 229 ($C_{14}H_{13}O_3$ as determined by high resolution mass spectrometry) represents a loss of CHO from the molecular ion. This fragmentation is best explained by a rearrangement (Fig 4) which has been proposed by Djerassi[9] for aliphatic epoxides and utilized previously in alloimperatorin methyl ether epoxide. The ion **23** would be highly stabilized thereby explaining its abundance. Peaks at *m/e* 214, 189, 159 and 131 are best explained by the sequence pictured in Fig. 4, a sequence previously reported, in part, for prenylated coumarins.[16]

A literature survey revealed that the structure **19** proposed for this natural product represented a new system, although a similar coumarin, phebalosin (**24**), in which the same side chain is found, has been recently reported.[17]



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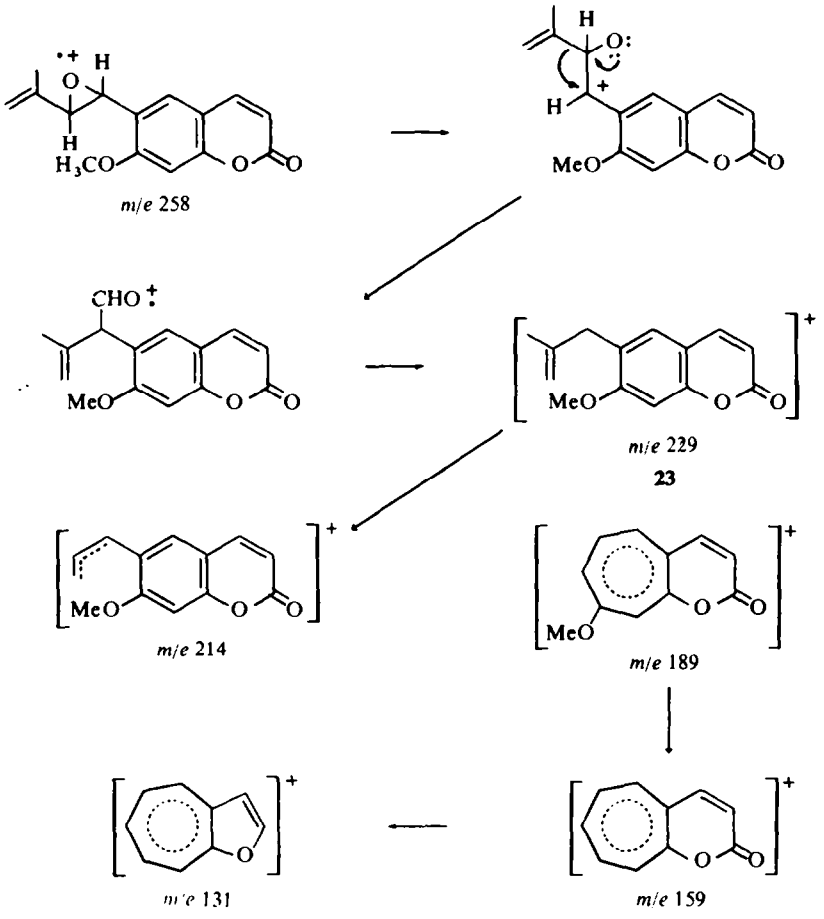
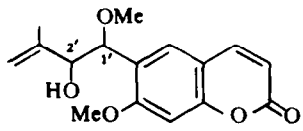


FIG 4. Proposed fragmentation of thamnosmin (**19**) in the mass spectrometer.

Phebalosin (**24**) and thamnosmin represent the only reported examples of coumarins with this unique side chain. The spectral data reported for **24** compared favourably with that of thamnosmin.

To further confirm the presence of the epoxide system in thamnosmin, an acid catalyzed ring opening reaction was undertaken. Treatment of **19** with HCl in MeOH aq yielded a crystalline product (40% yield) which on the basis of the following data could be assigned the structure **25**. High resolution mass spectrometry determined the molecular formula to be $C_{16}H_{18}O_5$. The UV spectrum was essentially unchanged from that of the starting material indicating the continued presence of the 6-alkyl-7-methoxycoumarin chromophore while the IR spectrum showed a sharp absorption at 3495 cm^{-1} readily assigned to a new hydrogen bonded hydroxyl group. The NMR spectrum was most helpful in the structural assignment. Signals in the aromatic region were essentially unchanged from the NMR spectrum of thamnosmin, except that the H(5) singlet was shifted downfield (0·19 ppm). Similarly the vinyl methyl and aromatic OMe group signals were unchanged. The remainder of the spectrum,



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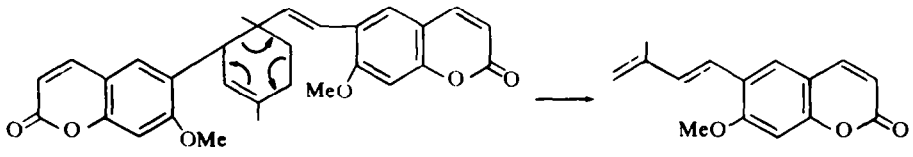
however, was significantly different. A doublet at τ 7·16 (J 3·2 Hz) which disappeared on addition of D_2O was assigned to the hydroxyl proton. A three proton singlet at τ 6·73 was readily assigned to the C(1') OMe group and a broad doublet at τ 5·96 (J 6·5 Hz) which became a sharp doublet on addition of D_2O, was assigned to H(2'). A three proton multiplet in the region τ 5·2–5·4, could be seen under high resolution to be composed of three multiplets, centered at τ 5·27 and 5·36 (assigned to the olefinic proton signals) and at τ 5·30 (assigned to H(1')).

The mass spectrum of this product offered conclusive evidence that epoxide opening had occurred as expected. Thus the characteristic peaks at *m/e* 189, 159 and 131 were again evident, indicating the unchanged nature of the basic skeleton. However, the molecular ion peak was now at *m/e* 290, with a peak at *m/e* 258 (M-MeOH). The base peak was now found at *m/e* 219. This peak is particularly significant as it represents a loss of C_4H_7O and this loss confirms that the epoxide has opened with introduction of the OMe function in the benzylic position.

This new natural product has been named thamnosmin since its structure is closely related to that of diene **26** which would be produced by a retro Diels–Alder reaction of the co-occurring dimer thamnosin (**9**). Such a process is actually observed in the mass spectrum of thamnosin.[3,4]



9 26

The co-occurrence of thamnosmin and thamnosin within the same plant raises the question of whether they are biogenetically related in that the diene **26** or the epoxide **19** could well serve as the monomeric template in the biosynthesis of **9**. This question is presently under investigation in our laboratory.

EXPERIMENTAL

M.ps were determined on a Kofler block and are uncorrected. Merck silica gel G (acc. to Stahl) with 2% fluorescent indicator added, was used as adsorbent in TLC unless otherwise noted. TLC plates were activated in an oven at 90° for one hr. For qualitative chromatography, layers of 0·3 mm thickness were used and spots were visualized by viewing under UV light. For preparative purposes large (20 × 20 cm) plates with a thicker layer (0·5 mm) were used. As with qualitative plates 2% fluorescent indicator was added and the bands visualized under UV light. Column chromatography was performed on either Shawinigan basic alumina (pH 10) deactivated with 10% AcOH to the desired activity, for large scale separations, or on Woelm neutral alumina, deactivated to the desired activity by addition of water as directed by the manufacturers, for smaller scale separations. Materials to be chromatographed were

normally preadsorbed on 10% of the adsorbent to be used. This was accomplished by dissolving the compound mixture in $CHCl_3$, adding the adsorbent, removing the solvent under aspirator pressure with gentle heating, then drying to a free flowing powder, under high vacuum. The preadsorbed material was poured dry onto the remaining 90% of the adsorbent prepared in the desired solvent and elution begun immediately.

GLC was carried out on an Aerograph Autoprep, model 700 with helium as carrier gas. UV spectra were measured in MeOH utilizing Cary, models 11 or 14 or a Unicam, model SP 800 spectrophotometer. IR spectra were measured routinely on Perkin–Elmer model 137 or 710 spectrophotometers. Analytical or comparison spectra were recorded on Perkin–Elmer, model 20 or 457 spectrometers. The positions of absorption maxima are quoted in wave numbers (cm^{-1}). NMR were measured in $CDCl_3$ at room temperature. Spectra were obtained either at 60 MHz on Jeolco C-60, Varian A-60 or Varian T-60 spectrometers or at 100 MHz on Varian HA-100 or Varian XL-100 spectrometers. Time averaged sensitivity enhancement was obtained by using the HA-100 spectrometer with a JASCO, model JRA 5, 4K memory computer attachment. Line positions are given in the Tiers τ scale with TMS as internal standard. The integrated peak areas, multiplicity and proton assignments are indicated in parenthesis. Mass spectra were measured on an Associated Electrical Industries, MS 902 high resolution mass spectrometer. High resolution molecular weight determinations were determined on the MS 902 spectrometer. A Jasco, model ORD/UV 5 spectropolarimeter was used to measure the ORD curves using MeOH as solvent. Microanalyses were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia.

Processing of Thamnosma montana *shoots.* Shoots, leaves and seed pods of *Thamnosma montana* plants were air dried and ground to a coarse powder. The ground material (250 g) was extracted with acetone (2 l), *via* soxhlet, and the acetone extract was evaporated *in vacuo* to yield an oily residue which was treated with $CHCl_3$ (500 ml). The $CHCl_3$ soluble portion was filtered and reduced to yield an oily residue (25 g) which, after preadsorption, was chromatographed on basic alumina (Activity IV–V, 1000 g). Elution with pet–ether and pet–ether–benzene yielded waxes. Further elution with benzene yield a fraction (0·6 g) which after prep TLC (anhydrous ethane–hexane, 1:1) provided umbelliprenin (10) (100 mg). Further elution with benzene provided a fraction (0·5 g) in which a component, isoimperatorin (**12**) was evident but not isolated. Further elution with benzene yielded a fraction (1·25 g) which after further chromatography on TLC (eluting with anhydrous ether–hexane, 1:1) provided alloimperatorin methyl ether (**7**) (380 mg), m.p. 113–114° (EtOAc) (lit.[2] m.p. 108–110°) in m.m.p. with authentic **7**, 108–110°. Also evident in this fraction, was isoimperatorin (**12**), thamnosmin (**19**) and phellopterin (**13**) and possibly bergapten (**15**), psoralen (**14**) and xanthotoxin (**16**) in small quantities (TLC inspection). Elution with $CHCl_3$–benzene provided a fraction (1·1 g) which on crystallization (EtOAc) yielded isopimpinellin (**2**) (450 mg), m.p. 149–151° (lit.[1] m.p. 148–149°), m.m.p. with authentic isopimpinellin 149–151°. Elution with $CHCl_3$ provided a fraction 2·0 g which after prep. TLC (eluting with EtOAc–$CHCl_3$ (1:1)) provided 5(2',3'-epoxy-3-methylbutyl)-8-methoxypsoralen (**11**) (350 mg) m.p. 103–104° (EtOAc–hexane). Further elution with MeOH yielded complex mixtures with no major components.

Properties of umbelliprenin (**10**). Umbelliprenin crystallized as white needles (from petroleum ether), m.p. 61–62° (lit.[6] m.p. 61–63°) IR (KBr) 1716, 1610, 1580 (α-pyrone), 895 (1,2,4-trisubstituted benzene), 835 ($R_2C{=}CHR$); λ_{max}^{MeOH} (ε) 206·5 (29,600), 251 (sh) (2500), 323 (15,700); NMR (100 MHz) $CDCl_3$, 2·47 (1H, d, *J* 9·5 Hz, H(4) of coumarin), 2·72 (1H, broad d, *J* 9·5 Hz, H(5), of coumarin), 3·20 (2H, m, H(6) and H(8) of coumarin), 3·85 (1H, d, *J* 9·6 Hz H(3) of coumarin), 4·56 (1H, broad t, *J* 6 Hz, $H_2C{-}\underline{CH}{=}C$), 4·93 (2H, broad m, $H_2C{-}\underline{CH}{=}C$), 5·44 (2H, d, *J* 6·0 Hz, $O{-}\underline{CH_2}{-}CH{=}C$), 7·8–8·2 (8H, methylene envelope), 8·26, 8·36 (6H, two s, two vinyl $\underline{CH_3}$), 8·43 (6H, s, two vinyl $\underline{CH_3}$); *m/e* 366, 204, 162, 134, 106, 105. Calcd. for $C_{24}H_{30}O_3$: C, 78·65; H, 8·25. Found: C, 78·52; H, 8·10%).

Synthesis of umbelliprenin (**10**).[8,21] Commercial farnesol was examined by GLC (column: 30% carbowax 20M, on 60/80 mesh chromosorb W, 10 ft × ⅜ in, helium flow rate 172 ml/min, 210°) and shown to consist of three isomeric components at r.t. 9 min (6%), 11 min (32%) and 12 min (60%). Prep. GLC on the same column (230°) and isolation of the last half of the last peak allowed isolation of *trans, trans*-farnesol[19] (205 mg); NMR (60 MHz) $CDCl_3$, 4·60 (1H, broad t, *J* 3·3 Hz, $CH_2{-}\underline{CH}{=}CR_2$), 4·88 (2H, broad m, two $\underline{CH}{=}CR_2$), 5·95 (2H, d, *J* 3·3 Hz, $O{-}\underline{CH_2}{-}CH{=}CR_2$), 7·6–8·2 (9H, methylene envelope and $\underline{OH}$), 8·33, 8·40 (12H, two s of almost equal intensity, four vinyl $\underline{CH_3}$).

To *trans, trans*-farnesol (205 mg, 0·925 mmol) in dry ether (10 ml), at −78°, PBr_3 (0·096 ml, 0·960 mmol) was added slowly. The mixture was allowed to come to room temp. then stirred for 6 hr, washed with 5% $NaHCO_3$ aq (20 ml), dried (Na_2SO_4) and filtered. This solution was used in the next reaction.

Umbelliferone (162 mg; 1·00 mmol), purified by sublimation *was dissolved* in absolute EtOH (5 ml) and

added to a solution of sodium (25 mg; 1·09 mmol) in absolute EtOH (5 ml). Removal of solvent *in vacuo* yielded the white salt of umbelliferone. The salt was dissolved in dry DMF (10 ml) and to this was added the ethereal solution of farnesyl bromide (0·925 mmol, theoretical). Most of the ether was removed under red press and the mixture stirred at room temp under N_2 for 14 hr. Water (25 ml) was added and the solution hexane extracted (3 × 50 ml). The hexane phase was dried and the solvent removed to yield a colourless oil (260 mg). Prep TLC (anhydrous ether–hexane) allowed isolation of umbelliprenin (200 mg) which crystallized from light pet-ether as white needles (150 mg; 44%) m.p. 58–59°, m.m.p. with umbelliprenin (**10**) from *Thamnosma montana* 58–60°, IR and UV spectra identical. (Calcd. for $C_{24}H_{30}O_3$: C, 78·65; H, 8·25. Found: C, 78·42; H, 8·10%).

Properties of 5-(2',3'-*epoxy*-3'-*methylbutyl*)-8-*methoxypsoralen* (**11**). Epoxide **11** crystallized from benzene–hexane as coarse crystals, m.p. 103–104° (lit.[2] m.p. 105–106·5°); $[\alpha]_D^{23} = < +1°$; IR (KBr) 1717 (C=O), 1588 (C=C); λ_{max}^{MeOH} (ε) 221 (25,200), 244 (sh) (19,900), 251·5 (22,800), 266 (20,350), 305 (13,000); NMR (100 MHz) $CDCl_3$, 1·82 (1H, *J* 10 Hz, H(4) of coumarin), 2·33 (1H, d, *J* 2 Hz) of furanocoumarin), 3·11 (1H, d, *J* 2 Hz, H(6) of furanocoumarin), 3·64 (1H, d, *J* 10 Hz, H(3) of coumarin), 5·78 (3H, s, O$\underline{CH_3}$), 6·5–7·2 (3H, mult, J_{AB} 14·5 Hz J_{AC} 3 Hz, J_{BC} 7·5 Hz; benzylic methylene and epoxide proton), 8·51, 8·70 (6H, two, C(O)C($\underline{CH_3}$)$_2$); *m/e* 300, 285, 271, 257, 229, 201, 199, 186, 171, 158. (Calcd. for $C_{17}H_{16}O_5$: C, 67·99; H, 5·37. Found: C, 67·91; H, 5·48%). High resolution MW determination. (Calcd. for $C_{17}H_{16}O_5$: 300·100. Found: 300·102).

Synthesis of 5(2',3'-*epoxy*-3'-*methylbutyl*)-8-*methoxypsoralen* (**11**). A solution of alloimperatorin methyl ether (**7**) (53 mg; 0·187 mmol) in $CHCl_3$ (5 ml) was cooled in an ice bath and to it was added a solution of *m*-chloroperbenzoic acid (36 mg; 0·204 mmol) in $CHCl_3$ (5 ml). The mixture was maintained at 0° for 5 hr, washed with 5% Na_2CO_3 aq (20 ml), and H_2O water, dried (Na_2SO_4) and the extract chromatographed on prep TLC (EtOAc–$CHCl_3$, 1:1) and 5(2',3'-epoxy-3'-methylbutyl)-8-methoxypsoralen (**11**) was isolated (47 mg; 84%) and crystallized from benzene–hexane to yield coarse white crystals of **11**, m.p. 103–104°, m.m.p. with the natural epoxide **11**, 114–116°, IR superimposable on that of the natural product.

Processing of Thamnosma montana *roots*. Air dried *Thamnosma montana* roots (400 g) were ground to a coarse powder and extracted *via* soxhlet with acetone (3 l) for 8 hr. The acetone extract was reduced to dryness and the oily extract $CHCl_3$ washed (1 l) and the resultant solution filtered, dried and evaporated *in vacuo* to yield a crude extract (30 g) which was preadsorbed and chromatographed on basic alumina (1000 g; activity III–IV). Elution with petroleum ether and benzene–petroleum ether provided a fraction (1·6 g) which contained waxes and nonpolarmaterials. The fraction eluted with benzene (0·73 g) was crystallized from MeOH to yield β-sitosterol (**3**) white crystals (20 mg), m.p. 134–137° (lit.[2] m.p. 137–139°) m.m.p. with authentic* β-sitosterol (**3**), 135–139°. The fractions eluted with $CHCl_3$–benzene and $CHCl_3$ (3·60 g) contained alloimperatorin methyl ether (**7**), γ-fagarine (**5**) and several new components observed in the shoots extract and thus they were combined for further column chromatography. The fraction eluted with acetone–$CHCl_3$ (5·79 g) was crystallized (EtOAc) to yield N-methylacridone (**4**) (300 mg) as yellow needles, m.p. 199–202° (lit.[2] m.p. 202–203°), m.m.p. with authentic* N-methylacridone (**4**), 200–203°. Prep TLC on 500 mg of the mother liquors (eluting with ether hexane 1:1 (3x)) from this crystallization allowed isolation of skimmianine (**6**), which was crystallized from EtOAc–hexane as a white powder (20 mg), m.p. 171–174° (lit.[2] m.p. 173–175°), m.m.p. with authentic skimmianine,* 171–174°. Thamnosin (**9**) was also present in this fraction but was more abundant in the next fractions, eluted with acetone–$CHCl_3$ and acetone (2·43 g). Prep TLC on this fraction (EtOAc–$CHCl_3$) allowed isolation of thamnosin (**9**) which crystallized (EtOAc) as colourless crystals (200 mg), m.p. 243–246° (lit.[2] m.p. 244–246°), m.m.p. with authentic thamnosin (**8**)[3,4] 243–246°.

The $CHCl_3$–benzene and $CHCl_3$ fractions (3·60 g) mentioned earlier were preadsorbed and chromatographed on Woelm alumina (neutral, 150 g, activity II). Elution with petroleum ether and benzene–pet ether yielded waxy material. Further elution with benzene–pet–ether yielded a fraction (685 mg) which on chromatography by prep TLC (eluting with anhyd ether–hexane) yielded isoimperatorin (**12**) (100 mg). Further elution with benzene and $CHCl_3$–benzene yielded fractions (total weight 932 mg) which after prep TLC (anhydrous ether–hexane (1:9), three elutions) provided alloimperatorin methyl ether (**7**), thamnosmin (**19**) (64 mg), phellopterin (**13**) (100 mg), psoralen (**14**) 30 mg) and bergapten (**15**) (110 mg).

Further elution with $CHCl_3$–benzene yielded a fraction (304 mg) from which xanthotoxin (**16**) (40 mg) was isolated *via* prep TLC (multiple elution with anhydrous ether–hexane (1:1)).

* Our gratitude is expressed to Dr. D. L. Dreyer for providing us with this sample.

Finally, elution with $CHCl_3$–benzene and $CHCl_3$ yielded a fraction (893 mg) which after prep TLC (eluting with EtOAc–$CHCl_3$) yielded γ-fagarine (**5**) (400 mg). m.p. 142·0–143·5° (lit.[2] m.p. 140–142°), m.m.p. with authentic γ-fagarine,[2] 140–142°.

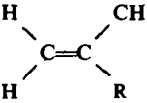
Properties of isoimperatorin (**12**). Isoimperatorin (**12**) crystallized from EtOAc–hexane as needles m.p. 97·0–98·0°, reforming plates m.p. 105–108° (lit.[13] m.p. 109°); IR (KBr) 1718, 1612, 1582 (α-pyrone), 819; λ_{max}^{MeOH} (ε) 219 (27,800), 249 (18,800), 257·5 (16,700), 267 (15,900), 308 (14,100); NMR (100 MHz) $CDCl_3$, 1·90 (1H, dd, *J* 10·0 Hz and 0·5 Hz, H(4) of furano-coumarin), 2·48 (1H, d, *J* 2·4 Hz, H(7) of furanocoumarin), 2·92 (1H, dd, *J* 0·5 Hz and 1·0 Hz, H(8) of furanocoumarin), 3·81 (1H, d, *J* 10·0 Hz, H(3) of furanocoumarin), 4·51 (1H, broad t, *J* 3·5 Hz, CH_2—$\underline{CH}$=CR_2), 5·13 (2H, d, *J* 3·5 Hz, O—$\underline{CH_2}$—CH=C), 8·22, 8·30 (6H, two s, C=$C(CH_3)_2$); *m/e* 270, 202, 174, 145, 118, 89, 67. (Calc. for $C_{16}H_{14}O_4$: C, 71·08; H, 5·22. Found: C, 70·90; H, 5·04. High resolution MW. (Calc. for $C_{16}H_{14}O_4$: 270·089. Found: 270·090).

Properties of phellopterin (**13**). Phellopterin (**12**) crystallized from EtOAc–hexane as coarse crystals m.p. 96–98° (lit.[20] m.p. 102°), m.m.p. with authentic phellopterin,[2] 95–98°; IR (KBr) 1730, 1606, 1593 (α-pyrone), 820 (RCH=CR_2); λ_{max}^{MeOH} (ε) 223 (25,800), 241 (14,600), 248 (14,600), 268·5 (16,700), 312 (11,500); NMR (100 MHz) $CDCl_3$, 1·95 (1H, d, *J* 10 Hz, H(3) of furanocoumarin), 2·45 (1H, d, *J* 2·2 Hz, H(7) of furanocoumarin), 3·10 (1H, d, *J* 2·2 Hz, H(6) of furanocoumarin), 3·79 (1H, d, *J* 10 Hz, H(4) of furano-coumarin), 4·44 (1H, broad t, *J* 7 Hz, CH_2—$\underline{CH}$=CR_2), 5·20 (2H, d, *J* 7 Hz, O$\underline{CH_2}$—CH=C), 5·88 (3H, s, aromatic O$\underline{CH_3}$), 8·29, 8·32 (6H, two s, two vinyl $\underline{CH_3}$), *m/e* 300, 285, 270, 232, 217, 189, 161, 133. (Calcd. for $C_{17}H_{16}O_5$: C, 67·99: H, 5·37. Found: C, 67·98; H, 5·36%).

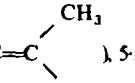
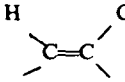
Properties of psoralen (**14**). Psoralen (**14**) crystallized from EtOAc–hexane as colourless needles, m.p. 173·5–164·5° (lit.[12] m.p. 163°); m.m.p. with authentic psoralen,* 163–164·5°; IR (KBr) 1714, 1630, 1574 (α-pyrone); λ_{max}^{MeOH} (ε) 211 (17,900), 240 (23,800), 245 (24,600), 298 (11,000), 325 (6,600); NMR (100 MHz) $CDCl_3$, 2·27 (1H, dd, *J* 9·5 Hz and 0·5 Hz, H(4) of furanocoumarin), 2·37, 2·39 (2H, respectively, d, *J* 2 Hz, H(7) of furanocoumarin and s, H(5) of furanocoumarin), 2·59 (1H, m, H(8) of furanocoumarin), 3·22 (1H, dd, *J* 2·0 Hz and 1·0 Hz, H(6) of furanocoumarin); *m/e* 186, 158, 130, 102. (Calc. for $C_{11}H_6O_3$: C, 70·97; H, 3·25. Found: C, 70·65; H, 3·15%).

Properties of bergapten (**15**). Bergapten (**15**) crystallized from EtOAc–hexane as colourless needles, m.p. 186·5–188·5° (lit.[12] m.p. 191°); m.m.p. with authentic bergaten* 186–188°; IR (KBr) 1726, 1623, 1580 (α-pyrone); λ_{max}^{MeOH} (ε) 221 (21,400), 248 (17,800), 257·5 (16,200), 267 (17,500), 309 (14,700); NMR (100 MHz), $CDCl_3$, 1·91 (1H, dd, *J* 9·75 Hz and 0·6 Hz, H(4) of furanocoumarin), 2·47 (1H, d, *J* 2·5 Hz, H(7) of furano-coumarin), 2·92 (1H, m, H(8) of furanocoumarin), 3·05 (1H, d, *J* 9·75 Hz, H(3) of furanocoumarin), 5·80 (3H, s, aromatic O$\underline{CH_3}$); *m/e* 216, 201, 188, 173, 145. (Calc. for $C_{12}H_8O_4$: C, 66·67; H, 3·73. Found: C, 66·57; H, 3·80%).

Properties of xanthotoxin (**16**). Xanthotoxin (**16**) crystallized from EtOAc–hexane as needles m.p. 146–147° (lit.[12] m.p. 146°); m.m.p. with authentic xanthotoxin* 140–142°; IR (KBr) 1710), 1615, 1582 (α-pyrone); λ_{max}^{MeOH} (ε) 218 (20,700), 247 (20,700), 261 (sh), (13,200), 300·5 (11,450); NMR (100 MHz) $CDCl_3$, 2·27 (1H, d, *J* 9·75 Hz, H(4) of furanocoumarin), 2·34 (1H, d, *J* 2·3 Hz, H(7) of furanocoumarin), 2·69 (1H, s, H(5) of furanocoumarin), 3·21 (1H, d, *J* 2·3 Hz, H(6) of furanocoumarin), 3·68 (1H, d, *J* 9·75 Hz, H(3) of furanocoumarin), 5·75 (3H, s, aromatic O$\underline{CH_3}$); *m/e* 216, 201, 188, 173, 145. (Calc. for $C_{12}H_8O_4$: C, 66·67; H, 3·73. Found: C, 66·31; H, 3·46%).

Properties of thamnosmin (**19**). Thamnosmin (**19**) crystallized from EtOAc–hexane as colourless plates, m.p. 101–104°; $[\alpha]_D^{23}$ ($CHCl_3$) = −17·3°; ORD (MeOH, *c*, 0·00106 $[\phi]_{219}$ −101,000°; IR (KBr) 1722, 1610, 1552 (α-pyrone), 3075, 903 (terminal methylene); λ_{max}^{MeOH} (ε) 227·5 (20,100), 253 (sh) (6,420), 297 (sh), (7,500), 327 (13,300); NMR (100 MHz) $CDCl_3$, 2·42 (1H, d, *J* 9·4 Hz, H(4) of coumarin), 2·77 (1H, broad s, H(5) of coumarin), 3·25 (1H, s, H(8) of coumarin) 4·85 (1H, m, width at half height 4 Hz, ), 5·93 (1H, dd, *J* 2·0 Hz and 0·65 Hz, benzylic epoxide proton), 6·13 (3H, s, aromatic O$\underline{CH_3}$), 6·80 (1H, broadened d, *J* 2·0 Hz, allylic epoxide proton), 8·25 (3H, dd, *J* 1·5 Hz and 0·9 Hz, H_2C=$\underline{CH_3}$); double irradiation NMR, (100 MHz), irradiate at 8·25, 4·85 m becomes apparent dd, 4·97 q becomes d (*J* 1·5 Hz); irradiate at 5·93, 6·80 d becomes s; irradiate at 1·77, 5·93 dd becomes d (*J* 2·0 Hz); *m/e* 258, 229, 214, 189, 159, 131. (Calc. for $C_{15}H_{14}O_4$: C, 69·75; H, 5·46. Found: C, 69·93; H, 5·71%). High resolution MW. (Calc. for $C_{15}H_{14}O_4$: 258·089. Found: 258·089. Calc. for $C_{14}H_{13}O_3$: 229·086. Found: 229·087).

* Our gratitude is expressed to Dr. D. L. Dreyer for providing us with this sample.

Acid hydrolysis of thamnosmin (**19**) *in methanol.*[19] Thamnosmin (**19**) (20·1 mg; 0·081 mmol) in MeOH (3 ml) was refluxed with H_2SO_4 (10%, 2·2 ml) for 4 hr. The mixture was poured into water and $CHCl_3$ extracted (3 × 20 ml). The extract was water washed, dried (Na_2SO_4) and evaporated under red press to yield a residue which was subjected to prep TLC (eluting with EtOAc–$CHCl_3$, 1:1) to yield a major component (8·4 mg), the MeOH adduct (**25**) which crystallized from benzene–hexane as colourless needles, m.p. 121·5–124·0°; IR (KBr) 3495 (OH), 3080, 905 (CH_2=CR_2), 1727, 1616, 1562 (α-pyrone); λ_{max}^{MeOH} (ε) 228·5 (16,100), 243 (sh) (5,630), 252 (4,425), 297·5 (sh) (7,450), 326 (12,800); NMR (100 MHz) $CDCl_3$ (expansions obtained *via* time averaging); 2·37 (1H, d, *J* 9·5 Hz, H(4) of coumarin), 2·58 (1H, s, H(5) of coumarin), 3·25 (1H, s, H(8) of coumarin), 3·76 (1H, d, *J* 9·5 Hz, H(3) of coumarin), 5·27 (1H, apparent q, *J* 1·5 Hz, ), 5·31, 5·32 (2H, superimposed, dd, *J* 6·4 Hz and 0·6 Hz, benzylic proton and m, ), 5·96 (1H, broad d becomes sharp on addition of D_2O, *J* 6·6 Hz, carbinolic proton), 6·16 (3H, s, aromatic O<u>CH</u>$_3$), 6·73 (3H, s, aliphatic O<u>CH</u>$_3$), 7·16 (1H, d, disappears on addition of D_2O, *J* 3·2 Hz, O<u>H</u>), 8·34 (3H, m, vinyl <u>CH</u>$_3$); *m/e* 290, 258, 219, 189, 159, 131. High resolution MW: Calc. for $C_{16}H_{18}O_5$: 290·115. Found: 290·114).

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