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CONSTITUENTS OF *THAMNOSMA MONTANA* TORR. AND FREM.

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Abstract—The structure of the toxic plant-growth-inhibitor occurring in *Thamnosma montana* Torr. and Frem. was shown to be 5-(3′-methyl-2′,3′-dihydroxybutanyl)-8-methoxypsoralen (I), largely by spectroscopic means. Racemic I was synthesized from alloimperatorin in three steps. In addition to the previously reported psoralens, isopimpinellin and byakangelicin it was found that alloimperatorin methyl ether, skimmianine, γ-fagarine and N-methylacridone also occur in this plant. The isolation and characterization of a coumestan derivative is also described.

THE turpentine broom, *Thamnosma montana* Torr. and Frem. (Rutaceae), is of special interest as a source of a series of three coumarins which show plant-growth-inhibitor properties[2,3] and on account of its use in American Indian folk medicine.[4]

Bennett and Bonner[2] isolated byakangelicin (VII), isopimpinellin (VI) and a third coumarin, m.p. 175–177·5°, $C_{16}H_{15}O_5(OMe)$. This latter material showed the highest toxicity of the three and was suggested to be an isobergapten derivative, although its UV spectrum is strikingly similar to alloimperatorin methyl ether (II).[5] This fact, when considered with the reported analytical data and with due regard for a biogenetically acceptable system, suggested that it was best represented as 5-(3′-methyl-2′,3′-dihydroxybutanyl)-8-methoxypsoralen (I).

In this study it was found that I was more conveniently isolated by chromatography than by the original procedures[2] and in the latter stages of the work it was found that I would sometimes crystallize directly from the benzene extracts of the defatted plant material. The UV spectrum of I could be superimposed on that of alloimperatorin methyl ether (II). The NMR spectrum of I confirmed the suggested structure. In addition to the usual psoralen resonances[6] the NMR spectrum showed the presence of a methoxy group at 250 c/s and a resonance for two C-methyl groups at 78 c/s displaced slightly downfield for C-methyls in a saturated system. This suggested a single bond oxygen function on the same carbon atom. Compound I formed a monoacetate (IV) under mild conditions which still showed hydroxyl absorption in the IR. The position of the hydroxyl band at 3560 cm^{-1} was consistent with the presence of a tertiary hydroxyl group in IV. The UV spectrum of the monoacetate was unchanged from that of the parent diol (I) showing that the hydroxyl groups were nonphenolic.

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[2] E. L. Bennett and J. Bonner, *Amer. J. Botany* **40**, 29 (1953).

[3] W. H. Muller and C. H. Muller, *Amer. J. Botany* **43**, 354 (1956).

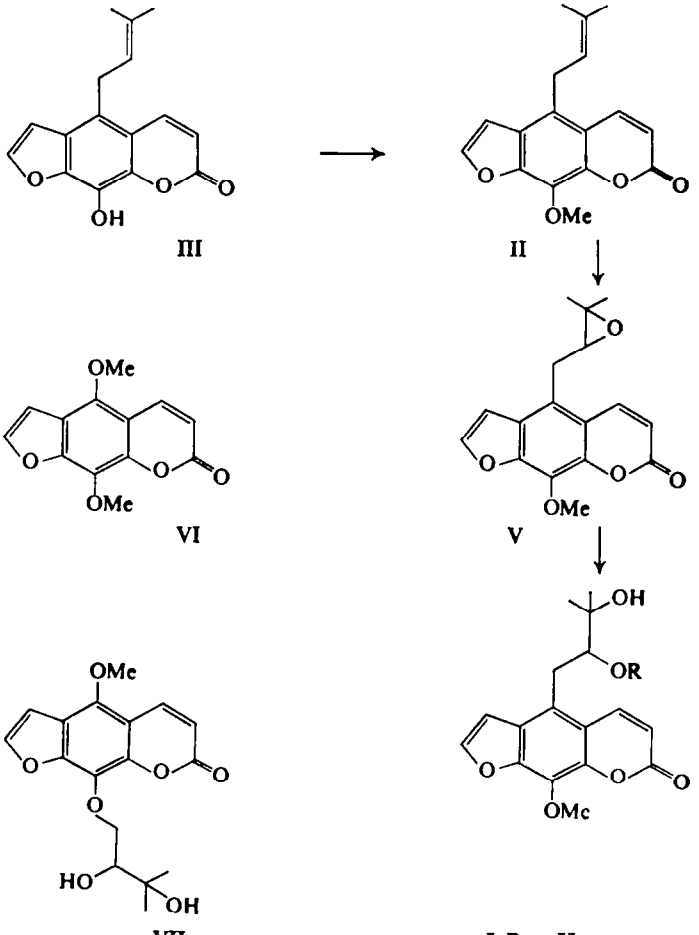
[4] T. H. Kearney and R. H. Peebles, *Arizona Flora* p. 494. University of California Press, Berkeley and Los Angeles, California (1960); L. Benson and R. A. Darrow, *A Manual of Southwestern Desert Trees and Shrubs* p. 210. University of Arizona, Tucson, Arizona (1945).

[5] D. L. Dreyer, *Phytochemistry* in press.

[6] See, for example, Ref. 5 and D. L. Dreyer, *J. Org. Chem.* **30**, 749 (1965).

The NMR spectrum of IV showed that the two C-methyls were nonequivalent. Acetylation thus appeared to appreciably restrict rotation about the isopentanyl 2,3 bond. The resonance of the 2'-proton of the acetate was a quartet and the benzylic protons gave rise to a complex multiplet, which appeared to be the AB part of an ABX pattern. This feature is diagnostic for a methylene attached to an asymmetric center.[7] Confirmation of these structural proposals was obtained by synthesis of I from alloimperatorin (III). Methylation of III under standard conditions gave the methyl ether (II).[8] Epoxidation of II gave V, which was hydrated to give racemic material, identical with natural I by spectroscopic criteria and TLC.

The structure of I suggested that the intermediates in its synthesis were also reasonable biogenetic intermediates in the plant. With the aid of the synthetic materials as reference samples the fractions from the chromatogram of the plant extracts were re-examined for the presence of compounds II and V by TLC. Among the nonpolar



[7] See, for example, G. M. Whitesides, D. Holtz and J. D. Roberts, *J. Amer. Chem. Soc.* **86**, 2628 (1964), R. K. Hill and T. Chan, *Tetrahedron* **21**, 2015 (1965) and Refs. contained therein.

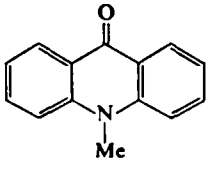
[8] E. Spaeth and H. Holzen, *Chem. Ber.* **66B**, 1137 (1933).

fractions was found material which corresponded to II. Complete identity was shown by isolation and check of its physical properties. The oxide (V) does not appear to be a detectable component of the plant as judged by TLC.

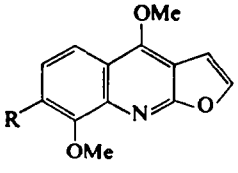
This is the third report of the alloimperatorin system occurring as a natural product. Alloimperatorin (III) itself has previously been reported as a component of *Poncirus trifoliata* seeds[8] and *Aegle marmelos* Correa,[9] both members of the Rutaceae.

In addition to the above psoralens, what is perhaps a coumarinobenzofuran derivative was also found in the less polar eluants of the column. This material, m.p. 244–246°, which we have chosen to call *thamnosin* analyzed for $C_{25}H_{28}O_5$ and showed no hydroxyl absorption in the IR. Thamnosin showed no optical activity at the sodium D-line and the general appearance of the UV spectrum suggested that it might be a coumesterol derivative with the long wavelength band shifted to slightly higher energies.[10] In addition, thamnosin showed a bright blue fluorescence under UV light, typical of this class of compounds. The NMR spectrum showed absorption bands for one normal C-methyl group in a saturated environment, one vinyl C-methyl, two methoxyls, one vinyl proton and a complex multiplet for nine aromatic protons. The presence of a lactone ring was shown by treatment with 5% NaOH in aqueous methanol and regeneration of the starting material upon acidification. Thamnosin decolarized bromine in chloroform and was oxidized by permanganate. Thamnosin was remarkably stable to strongly basic conditions, for example, hot 20% KOH. Fusion with potassium hydroxide did, however, give resorcinol.

Fractions eluted from the column with benzene–chloroform showed an intense blue fluorescence. These fractions yielded N-methylacridone (VIII) identical with a sample prepared by methylation of acridone. Workup of the chloroform eluants from the column gave skimmianine (IX) and γ-fagarine (X) which are typical rutaceous furoquinoline alkaloids.[11] These latter two alkaloids could be obtained from 15% HCl washings of the crude plant extracts. The N-methylacridone could also be obtained from the crude plant extracts by washing with 50% HCl. N-methylacridone has not previously been reported as a natural product and the isolation of the parent member of the acridone alkaloids[12] is rather surprising since most alkaloids of this series show highly substituted A-rings. Moreover, most of the other products from this plant are of a moderately high oxidation level.



VIII



IX, R = MeO
X, R = H

[8] S. K. Saha and A. Chatterjee, *J. Ind. Chem. Soc.* **34**, 228 (1957); *Chem. Abstr.* **51**, 16730 (1957).

[10] See, for example, L. Jurd, *J. Org. Chem.* **24**, 1786 (1959); T. R. Govindachari, K. Nagarajan and P. C. Parthasarathy, *J. Chem. Soc.* 548 (1957). See also UV data on some cinnamic acid derivatives reported by O. Halpern, P. Waser and H. Schmid, *Helv. Chim. Acta* **40**, 758 (1957).

[11] H. T. Openshaw in R. H. F. Manske and H. L. Holmes, *The Alkaloids, Chemistry and Physiology* Vol. II; p. 65. Academic Press, New York, N.Y. (1952).

[12] J. R. Price, Ref. 10, Vol. III; p. 353.

EXPERIMENTAL[13]

Isolation. The whole aerial part of the plant including the crown, collected as needed between July and November from hillsides near Morongo Valley, California, was ground and extracted with acetone. The acetone extracts were concentrated to about ¼ volume, and the dark heavy tar which separated was removed by filtration through celite. Most of the solvent was removed from the filtrates and the dark heavy oily residue was taken up in $CHCl_3$ and dried. This material was chromatographed on acid-washed alumina. The content of the fractions was checked by TLC with silica gel absorbant and a 1:1 $CHCl_3$: AcOEt solvent system. The first fractions, eluted with hexane and hexane–benzene contained large amounts of fats and waxes. Direct crystallization of the residue of the hexane–benzene eluants from MeOH gave β-sitosterol, m.p. 137–139° undepressed with that of an authentic sample. Isopimpinellin was eluted with benzene and its identity shown by comparison with an authentic sample. The benzene eluants also gave thamnosin, which was crystallized from AcOEt or benzene to give colorless crystals, m.p. 244–246°; ν 1726, 1611, 1561 cm^{-1} (Nujol); λ_{max}^{EtOH} 203 (40,500), 228 (22,600), 256 (22,300), 298 (11,500), 335 (18,500) mμ. Thamnosin was not readily sublimable and was rather insoluble in alcohol or acetone but readily soluble in $CHCl_3$; mass spect. mol. wt. 406. (Found: C, 73·9; H, 5·83. $C_{25}H_{26}O_5$ requires: C, 73·87; H, 6·45%.)

The first fractions eluted with ca. 1:1 benzene: $CHCl_3$ showed an intense blue fluorescence in visible light. Removal of solvent from the intensely fluorescent fractions and crystallization of the residue from AcOEt gave lemon yellow crystals m.p. 202–203°. This material proved to be identical by mixed m.p. UV and IR spectra and R_f on TLC with a synthetic sample of VIII and was easily detected on TLC by emersing the plate in a tank of HCl gas to give bright yellow spots.

Workup of the $CHCl_3$ eluants from the column gave IX, m.p. 173–175°, from AcOEt, identical in all respects with an authetic sample.[14] Further crystallization of the mother liquors gave X, m.p. 140–142° from AcOEt–hexane.[14] The furoquinoline alkaloids could also be obtained directly from the $CHCl_3$-plant extracts by extraction with 15% HCl. Regeneration and crystallization from MeOH gave skimmianine. Removal of solvent from the mother liquors and crystallization of the residue from AcOEt–hexane gave γ-fagarine.

The diol (I) was obtained by further elution with 10% acetone in $CHCl_3$, m.p. 174–176° from AcOEt; $[\alpha]_D^{23°}$ −30·6° (95% EtOH); ν 3410 (hydroxyl), 1713, 1592 cm^{-1} (Nujol); λ_{max}^{EtOH} 220 (23,900), ~244 (15,500), 251 (17,000), 266 (15,500), 309 (11,400) mμ; NMR 495 (d) J = 10H-4, 465 (d) J = 2H-7, 420 (d) J = 2H-6, 376 (d) J = 10H-3, 250 (s) methoxy, 188 (d) J = 7 benzylic, 208 (t) J = 7 carbinol, 78 (s) C-methyls c/s ($CDCl_3$–deuteriodimethyl sulfoxide). (Found: C, 64·3; H, 5·63. $C_{17}H_{18}O_6$ requires: C, 64·14; H, 5·70%.)

After the initial isolation by chromatography, it proved possible to obtain I directly from benzene extracts of the defatted plant material after concentration and allowing the solution to stand several days.

The R_f of I and an authentic sample of byakangelicin on TLC were almost identical. Thus, successive crops of I were removed with EtOH–AcOEt. Solvent was then removed from the mother liquors and the residue crystallized from acetone to give byakangelicin, m.p. 105–107°. Comparison of its IR spectrum with that of an authetic sample established identity: ν 3306 (hydroxyl), 1717, 1592 cm^{-1} (Nujol); $[\alpha]_D^{23°}$ +34·7° (95% EtOH) NMR 488 (d) J = 10H-4, 465 (d) J = 2H-7, 426 (d) J = 2H-6, 375 (d) J = 10H-3, 270–258 (multiplet) ether methylene, 251 (s) methoxy, 230 (diffuse) carbinol proton, 76 (s) C-methyls c/s ($CDCl_3$–deuteriodimethyl sulfoxide). λ_{max}^{EtOH} 233 (24,000), 252 (11,100), 259 (12,000) 279 (14,800) 323 (9,500) mμ.

Extensive rechromatography of the hexane–benzene eluants gave alloimperatorin methyl ether,[8] m.p. 108–110°, from benzene–hexane. This material had IR and UV spectra and R_f on TLC identical with that from a synthetic sample prepared as described below.

Acetylation of I. Excess Ac_2O was added to 50 mg of I in pyridine and the mixture allowed to stand overnight. After workup, the product was crystallized from 95% EtOH to give 45 mg of IV, m.p. 183–185°, ν 3560 (hydroxyl), 1742, 1588 cm^{-1} (Nujol); λ_{max}^{EtOH} 220, ~244, 251, 266, 309 mμ;

[13] NMR spectra were taken at 60 Mc and the data are given in c/s relative to internal TMS. The relative areas of peaks were consistent with the assignments. The m.ps are uncorrected.

[14] For NMR data on skimmianine and γ-fagarine, see A. V. Robertson, *Austr. J. Chem.* **16**, 451 (1963); for other spectral data see L. H. Briggs and R. C. Cambie, *Tetrahedron* **2**, 256 (1958); J. Iriate, F. A. Kincl, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.* 4170 (1956).

NMR 488 (d) J = 10H-4, 461 (d) J = 2H-7, 416 (d) J = 2H-6, 383 (d) J = 10H-3, 302 (quartet) carbinol, 255 (s) methoxy, 200 (multiplet) benzylic protons, 105 (s) acetoxy, 82, 77 C-methyls c/s ($CDCl_3$). (Found: C, 63·5; H, 5·80. $C_{19}H_{20}O_7$ requires: C, 63·32; H, 5·59%.)

Alloimperatorin methyl ether (II)[5,6]. Compound III was conveniently methylated on a 10 g scale with MeI and K_2CO_3 in refluxing dry acetone for 24 hr. The reaction mixture was cooled, filtered, and solvent removed. The residue was crystallized from dil MeOH to give a 8 g of material identical with that prepared with diazomethane;[5] m.p. 106–109°. λ_{max}^{EtOH} 220 (20,400) ~244 (12,500), 250 (13,500), 266 (12,100), 308 (8,400) mμ.

Alloimperatorin methyl ether oxide (V). To a solution of 0·5 g of II in $CHCl_3$ was added 0·35 g *m*-chloroperbenzoic acid, and the solution was maintained at 0° for 48 hr. The solution was then extracted with 5% Na_2CO_3aq, dried, and solvent removed. The residue was filtered through a short column of acid-washed alumina with 1: 1 benzene–$CHCl_3$. Solvent was removed from the eluants, and the residue crystallized from benzene–hexane, m.p. 105–106·5°, 80% yield; ν 1722, 1587 cm^{-1} (Nujol); λ_{max}^{EtOH} 220 (26,800), ~244 (18,700), 251 (21,000), 266 (17,400), 308 (12, 900) mμ; NMR 489 (d) J = 10H-4, 464 (d) J = 2H-7, 418 (d) J = 2H-6, 383 (d) J = 10H-3, 255 (s) methoxy, 180–200 (multiplet) benzylic and epoxy protons, 90, 79 C-methyls c/s ($CDCl_3$). (Found: C, 68·1; H, 5·43. $C_{17}H_{16}O_5$ requires: C, 67·99; H, 5·37%.)

5-(3′-*Methyl*-2′,3′-*dihydroxybutanyl*)-8-*methoxypsoralen* (I). The oxide (V; 105 mg) was added to 30 ml boiling 5% aq oxalic acid solution and refluxing continued for 30 min. The solution was cooled and extracted with $CHCl_3$. The extracts were washed with 5% Na_2CO_3, dried and solvent removed to give a residue which was recrystallized from AcOEt. The product (73 mg) m.p. 173–174°, was identical by spectroscopic criteria and R_f on TLC on TLC with natural I.

N-*methylacridone*. To a solution of 9·8 g acridone in tetrahydrofuran was added 2·4 g of 50% NaH dispersion in oil with stirring. After the rapid H_2 evolution ceased, 6·3 ml dimethyl sulfate in tetrahydrofuran was added with stirring. After 20 min the mixture was poured into water and the product collected. Recrystallization several times from EtOH gave 6 g of product; m.p. 199–201° (lit[15] m.p. 203·5°); NMR 507 (quartet) (J = 8, J = 2) H-1, 443 (multiplet) aromatic, 230 (s), N-methyl c/s ($CDCl_3$). (Found: N, 6·64. $C_{14}H_{11}NO$ requires: N, 6·68%.)

Base fusion of thamnosin. Thamnosin (10 mg) was melted with two KOH pellets in a test tube and heated for 4 to 5 min. The mixture was cooled, diluted, acidified and extracted with AcOEt. Paper chromatography in two solvent systems indicated the presence of resorcinol. Removal of solvent and sublimation of the residue gave resorcinol.

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[15] C. Graebe and K. Lagodzinski, *Leibigs Ann.* **276**, 35 (1893).