Tetrahedron. Vol. 26, pp. 3171 to 3184. Pergamon Press 1970. Printed in Great Britain

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

THE STRUCTURE OF THAMNOSIN, A NOVEL DIMERIC COUMARIN SYSTEM*[1]

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(*Received in USA* 15 *December* 1969; *Received in the UK for publication* 9 *February* 1970)

Abstract—The structure determination of thamnosin, a minor component obtained from *Thamnosma montana* Torr. and Frem. is described. Thamnosin (VI) represents a novel dimeric coumarin system which was not previously encountered in nature.

THAMNOSMA montana Torr. and Frem. (Rutaceae), more commonly known as turpentine broom, has over the years attracted the interests of several groups of research workers. It represents a source of coumarins which exhibit plant-growth-inhibitor properties[2, 3] and its use in folk medicine is also reported.[4]

Previous investigations[2, 5] on the constituents of this plant have established the presence of a number of monomeric coumarins as well as a few furoquinoline alkaloids. In addition, in one of these reports,[5] a minor component, thamnosin, $C_{25}H_{26}O_5$, was isolated but no definitive structural assignment could be made. Evidence is now presented which illustrates that thamnosin possesses a unique dimeric coumarin system.

Re-examination of the molecular formula by high resolution mass spectrometry required a revision to $C_{30}H_{28}O_6$. The strong and complex absorption in the UV spectrum and in the appropriate regions of the NMR spectrum of thamnosin suggested the presence of a highly conjugated system. Bands at 1725, 1610 and 1557 cm^{-1} in the IR spectrum indicated the presence of α-pyrone or coumarin chromophores and clearly eliminated a γ-pyrone system from consideration.

The mass spectrum of thamnosin was very striking since there were virtually no peaks between the molecular ion (m/e 484, 8% abundance) and the base peak (m/e 242). This important result suggested that thamnosin was cleaved, under electron impact, into two equal halves, and therefore some structural symmetry must be present in this molecule. The detailed mass spectra of thamnosin and its derivatives, however, will be discussed in another publication.[6]

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

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The NMR spectrum of thamnosin (Fig 1) indicated the presence of a tertiary methyl (τ 8·78, probably allylic), a vinyl methyl (τ 8·20), two methoxyl groups (τ 6·29 and 6·27), an olefinic proton (τ 4·75, multiplet) and a complex multiplet in the aromatic region (τ 2·4–3·9, ten protons). An expansion of the latter region revealed the presence of a conjugated *trans*-disubstituted double bond (AB pattern at τ 3·98 and 3·82, J_{AB} = 16 Hz).

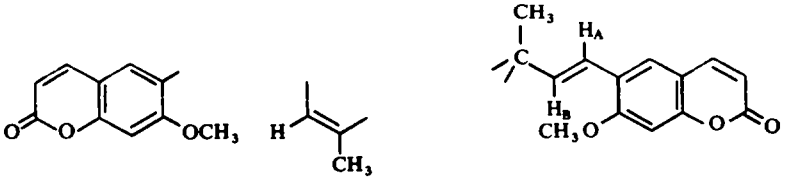
It was clear from the above molecular formula that seventeen degrees of unsaturation were present in thamnosin and therefore the first selected reaction was catalytic hydrogenation to investigate the nature of any double bonds which may be present.

Thamnosin in the presence of 10% palladium on charcoal in tetrahydrofuran smoothly absorbed one mole of hydrogen. The elemental analysis and the high resolution mass spectrum confirmed that this compound, hereafter called dihydrothamnosin, m.p. 226–228°, had the molecular formula $C_{30}H_{30}O_6$.

The IR spectrum of dihydrothamnosin showed very similar bands for the carbonyl and double bond stretching frequencies originally observed in thamnosin but no bands were present at 980 cm^{-1}. The olefinic resonances (AB pattern) originally present in thamnosin were now absent in the NMR spectrum of the dihydro derivative. The above spectral data confirmed that a *trans*-disubstituted double bond had been reduced in the molecule. It could now be further suggested from the chemical shift and the multiplicity pattern of this particular olefinic system that its linkage in thamnosin involved an aromatic portion on the one hand and a tertiary carbon atom on the other. The chromophoric change created by the hydrogenation reaction as shown in the UV spectrum [λ_{max}^{MeOH} 227, 256, 298 (sh), and 333 mμ in thamnosin; λ_{max}^{MeOH} 224, 246 (sh), 254, 300 (sh), and 330 mμ in dihydrothamnosin] confirmed the presence of a double bond conjugated to an aromatic system, the latter most likely being a coumarin chromophore. In fact, the virtual identity of the absorption maxima in the UV spectrum of the dihydro compound with that of suberosin (7-methoxy-6-isopent-2′-enylcoumarin)[7] dictated the presence of a 6-substituted 7-methoxycoumarin skeleton. Furthermore, the intensity of this absorption, being essentially *twice* that of suberosin, suggested that dihydrothamnosin consisted of two 7-methoxycoumarin moieties (C_{10} × 2) and a C_{10}-alkyl residue linked to the 6-position of these molecules.

The NMR spectrum of dihydrothamnosin again showed sharp singlets for a tertiary methyl (τ 8·97), a vinyl methyl (τ 8·26), two methoxyl resonances (τ 6·25 and 6·22), an olefinic proton (τ 4·83, multiplet), and a series of signals in the aromatic region (τ 2·4–3·9) which now integrated for eight protons. The significant upfield shift of the tertiary methyl (τ 8·78, thamnosin → τ 8·97, dihydrothamnosin) suggested that it was situated on a carbon atom adjacent to the reducible double bond.

On the basis of the above evidence it was possible to postulate, as a working hypothesis, the following partial structure (I) for thamnosin.



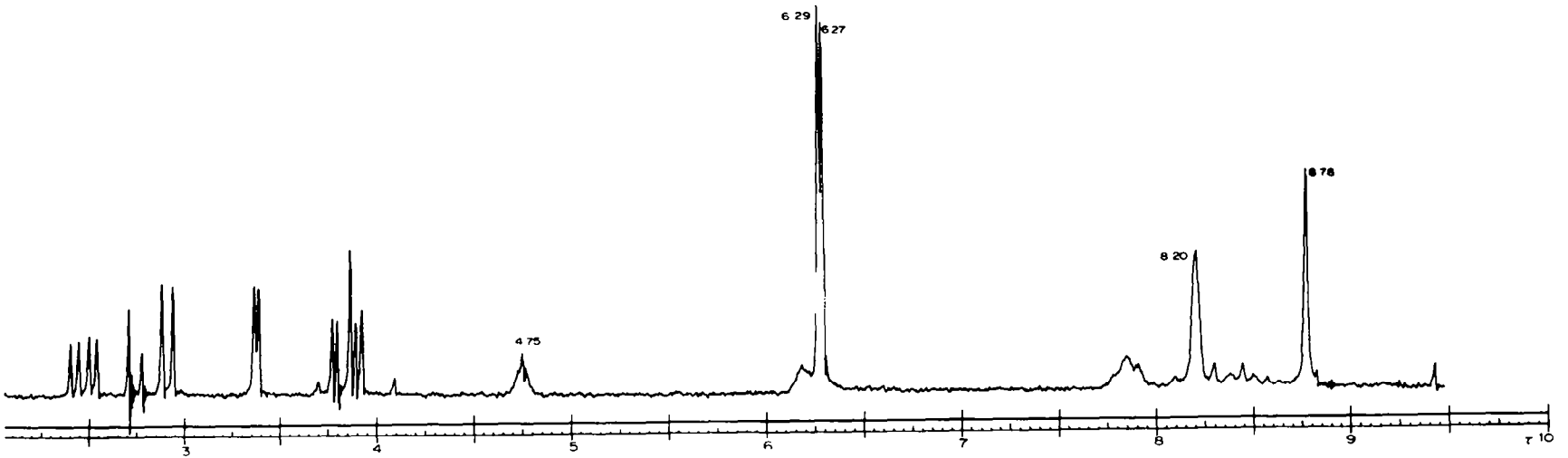


FIG. 1 NMR Spectrum of Thamnosin (100 MHz)

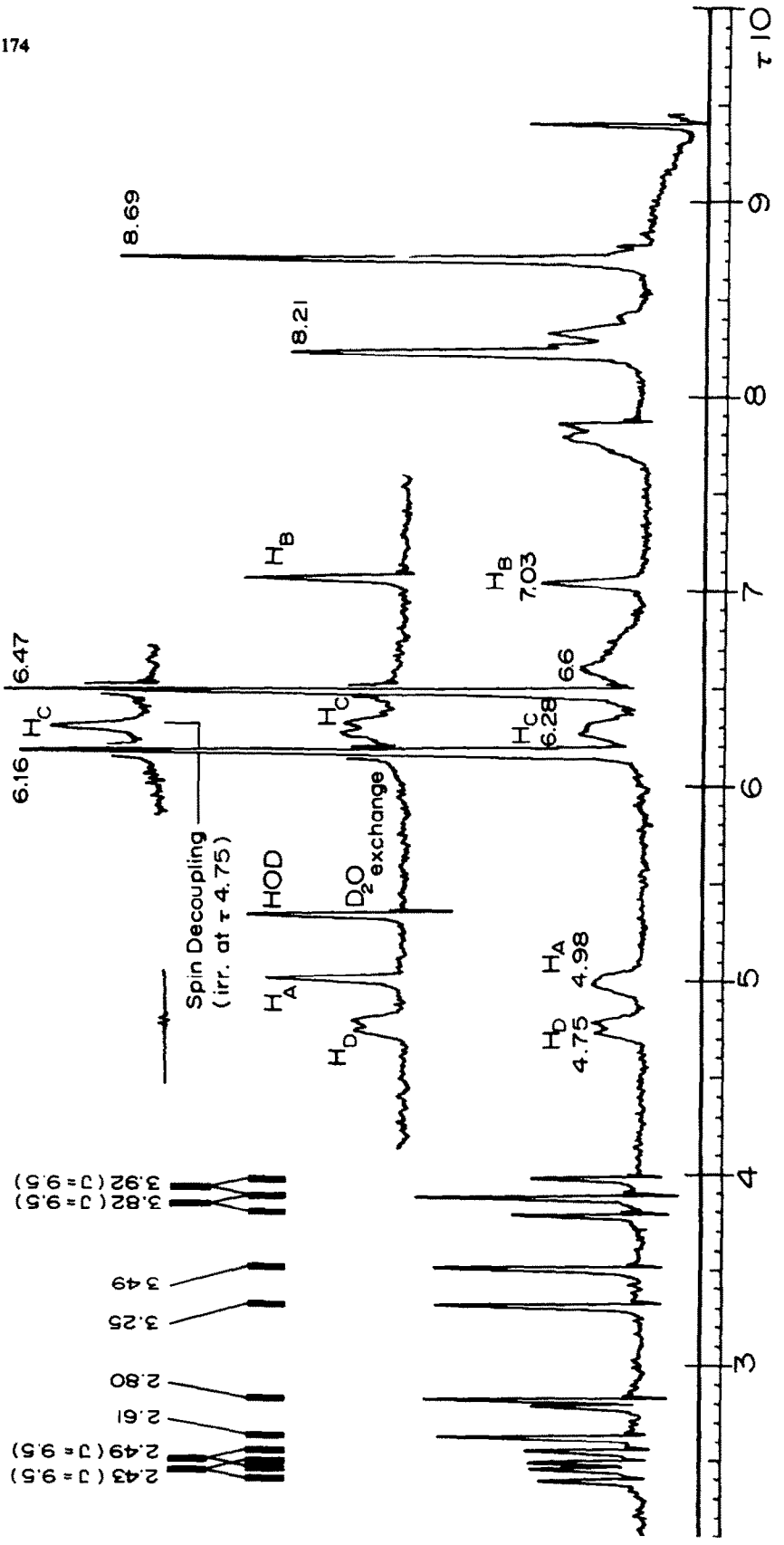
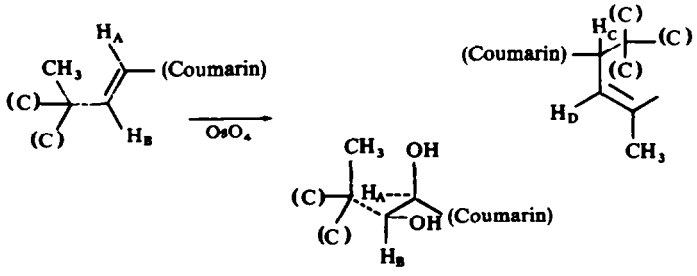


FIG. 2 NMR Spectrum of Thamnosindiol (100 MHz)

In order to obtain lower molecular weight fragments which may be more easily compared with compounds of known structure, the cleavage of thamnosin was next considered. The *trans*-disubstituted double bond in thamnosin was thought to be a convenient handle for this purpose and therefore its conversion to a diol was attempted.

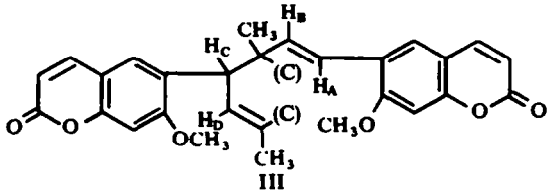
The successful hydroxylation of thamnosin was accomplished by treating it with osmium tetroxide to give thamnosindiol, $C_{30}H_{30}O_8$, which possessed a UV spectrum identical with that of dihydrothamnosin. As expected, the hydroxylation and the catalytic hydrogenation reactions were both proceeding on the same double bond of thamnosin.



II

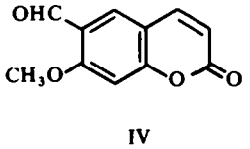
The NMR spectrum of thamnosindiol (Fig 2) was very instructive and clearly indicated the presence of all thirty protons. Apart from the usual signals already mentioned above one-proton singlets at τ 7·03 (H_B, see partial structures given in II) and τ 4·98 (H_A) and one-proton doublets at τ 6·28 (H_C, J = 5 Hz) and τ 4·75 (H_D, J = 5 Hz) as well as a two proton multiplet at τ 6·6(OH) were of significance. Spin decoupling experiments demonstrated that irradiation at the resonance frequency of the olefinic proton (τ 4·75) allowed the doublet at 6·28 to collapse into a singlet. This result, along with the chemical shift, indicated that H_C must be situated next to an aromatic system and a fully substituted aliphatic carbon atom. Addition of deuterium oxide sharpened the peaks at τ 7·03 and τ 4·98 and caused the two-proton multiplet at τ 6·6 to disappear. Virtually no couplings between H_A and H_B in the diol were observed after deuterium exchange and on this basis it appeared that the dihedral angle between H_A and H_B was close to 90°. A study of molecular models suggested that a *cis* hydroxylation of the postulated *trans* double bond would yield a diol with a dihedral angle between H_A and H_B of approximately 60°. According to Karplus[8] this should provide a small coupling constant (J = 2 Hz) between these protons. However, it must be remembered that substituents with high electronegativity are known to reduce the coupling constants of vicinal protons[8] and in this instance both H_A and H_B are attached to carbon atoms bearing hydroxyl groups.

Consideration of the above spectral evidence allowed us to expand the tentative structure of thamnosin to III.

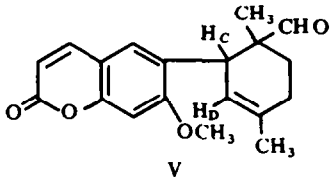


III

It was now desirable to attempt a cleavage of thamnosindiol into lower molecular weight fragments. For this purpose, the diol was reacted with periodic acid in aqueous methanol at room temperature. Two aldehydic compounds designated as aldehyde-I and aldehyde-II were obtained from this reaction. High resolution mass spectra of these aldehydes determined the molecular formulae as $C_{11}H_8O_4$ and $C_{19}H_{20}O_4$, respectively, and provided conclusive evidence that this reaction cleaves the diol into two compounds *without* any loss of carbon.



IV



V

Aldehyde-I had a complex UV absorption (λ_{max}^{MeOH} 255, 308 and 329 mμ) probably due to an extended conjugation of the coumarin system with the aldehyde group. When sodium borohydride was added to a methanolic solution of aldehyde-I and then the UV spectrum was recorded, the spectrum changed dramatically and was now almost superimposable on the spectra of dihydrothamnosin and suberosin. The hydroxymethyl group which would be derived from the aldehyde in the hydride reaction would not be expected to contribute significantly to the UV spectrum and it was not surprising that a typical 7-methoxycoumarin chromophore was now in hand. The IR spectrum of aldehyde-I still showed the presence of a coumarin system and the NMR spectrum indicated singlets at τ 6·01 (CH_3O—) and τ −0·23 (Ar—CHO). On the basis of this evidence the structure of aldehyde-I was proposed to be 7-methoxycoumarin-6-aldehyde (IV), a known degradation product from ostruthin[9] and suberosin.[7] The identity of these two compounds was established by a direct comparison with an authentic sample.[10]

Aldehyde-II similarly indicated in the UV spectrum, the presence of a 7-methoxycoumarin system bearing a C-6 alkyl side chain. This result confirmed the previous suggestion that the thamnosin molecule contained two coumarin chromophores most probably linked to a C_{10} alkyl side chain.

The NMR spectrum of aldehyde-II (Fig 3) was again very informative. Two sharp singlets at τ 8·82 and 8·21 confirmed the presence of a tertiary and a vinyl methyl group, respectively. In addition, a methoxyl signal at τ 6·20, a one-proton doublet at τ 5·84 (H_C, J = 5 Hz), a multiplet at τ 4·76 (H_D), a series of signals for four aromatic protons and a one-proton singlet at τ 0·73 for the saturated aldehyde proton were the remaining significant signals. The chemical shift of the tertiary methyl (τ 8·82) and the presence of a singlet for the aldehydic proton gave some evidence that both of these groups may be attached to the same fully substituted carbon atom. This suggestion was made earlier in comparing the NMR spectra of thamnosin and dihydrothamnosin. The significant downfield shift of H_C (τ 5·84) in aldehyde-II relative to thamnosin or its other derivatives must be due to its close proximity to the aldehyde group. Additional information about the four aromatic protons of aldehyde-II was obtained when the splitting patterns of the aromatic signals were examined in the NMR spectra taken at 60 MHz and 100 MHz. It turned out that this region consisted of two sets of doublets (τ 3·84, J = 9·5 Hz, H—C_3 of coumarin and 2·45, J = 9·5 Hz, H—C_4 of

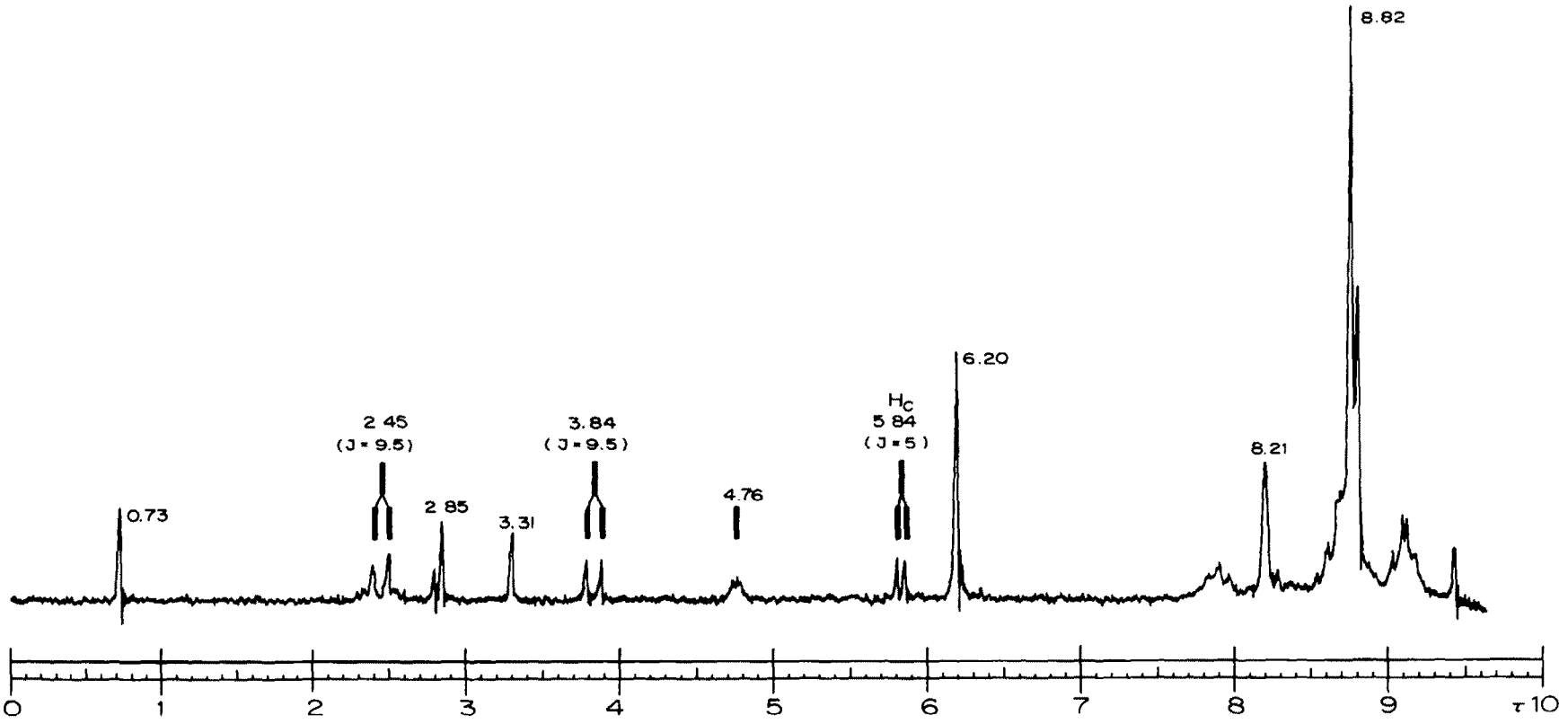
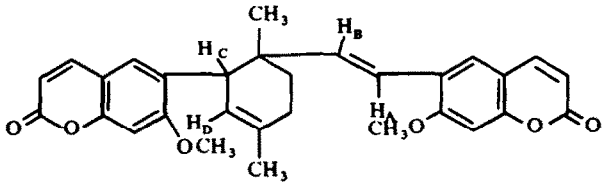


FIG. 3 NMR Spectrum of Aldehyde—II

coumarin) and two singlets (τ 3·31, H—C_8 of coumarin and 2·85, H—C_5 of coumarin) for which assignments were readily made. It must be noted that the aromatic regions of marmesin[11] and suberosin (see experimental) showed virtually the same patterns and chemical shifts as those of aldehyde-II. This NMR evidence showed without doubt, that aldehyde-II contained a 7-methoxycoumarin system with an alkyl side chain at C-6.

The above data, when taken in conjunction with the previous results (only C_2H_4 and one degree of unsaturation still remain unaccounted in thamnosin), establish structure V for aldehyde-II and, in turn, VI for thamnosin.

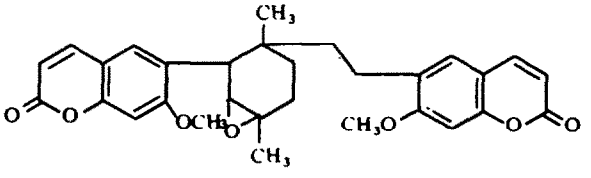


VI

The remaining portion of the discussion will be devoted to experiments which substantiate these proposals.

In order to provide evidence for the presence of the trisubstituted double bond in a cyclohexene system as postulated in V and VI, several reactions on dihydrothamnosin in which the conjugated, disubstituted double bond had been removed were considered.

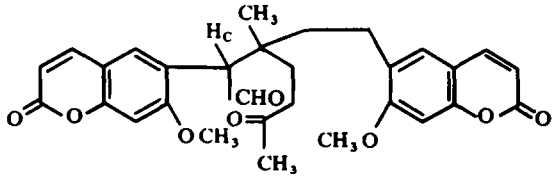
The epoxidation of dihydrothamnosin by means of *m*-chloroperbenzoic acid gave a single product whose high resolution mass spectrum and elemental analyses were in good agreement with the molecular formula of a mono-epoxide, $C_{30}H_{30}O_7$. The coumarin systems were shown to be still intact as indicated by the UV and IR spectra. The significant feature of the NMR spectrum was that one of the two methyl groups in the starting material had shifted significantly to higher field (τ 8·26 → 8·59) and that no olefinic proton was present in the molecule. This NMR evidence now confirmed the presence of the moiety, $CH_3-\overset{|}{C}=C\overset{\diagup}{\diagdown}$, in dihydrothamnosin. The NMR spectrum of dihydrothamnosinoxide (VII) still showed a sharp three proton singlet at τ 9·17, a single peak for two methoxyl groups at τ 6·16, and eight protons in the region, τ 2·5–3·9. On the basis of the above, it was clear that a straightforward epoxidation of the double bond was occurring.



VII

It was hoped that the epoxide could serve as an intermediate for subsequent degradation of the molecule. Unfortunately attempts to cleave the epoxide ring under a variety of conditions always led to a complex mixture of products.

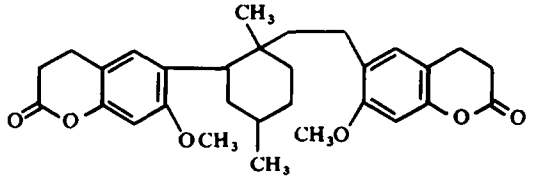
Coumarins are, however, known to be stable towards a very dilute stream of ozone and it was felt that this reaction might yield fruitful results. Indeed the controlled ozonolysis of dihydrothamnosin followed by catalytic reduction of the ozonide gave a single compound, designated as ketoaldehyde-III. The UV and IR spectra of this substance indicated the retention of the 6-alkyl-7-methoxycoumarin chromophore. In particular, NMR signals for the aldehydic proton (τ 0·02, doublet, J = 2 Hz), a methyl ketone (τ 7·87, singlet) and the proton H_C (see VIII) which appeared as a doublet (τ 5·76, J = 2 Hz) should be noted. The aldehydic proton shown in VIII was found to be coupled with a single proton (H_C) by spin decoupling experiments. Therefore it was now established that the carbon atom bearing H_C of thamnosin could only be connected to an aromatic system, a fully substituted carbon atom and a trisubstituted double bond whose olefinic proton as shown by previous decoupling experiments was in turn also coupled with H_C. When this evidence was taken in conjunction with the previous results, it was concluded that ketoaldehyde-III had the structure VIII.



VIII

A series of hydrogenation experiments were also conducted on dihydrothamnosin to establish the nature of the unsaturation present in this molecule. Tetrahydrothamnosin was obtained when the hydrogenation was interrupted after one mole of hydrogen had been adsorbed. The presence of coumarin systems was shown by the IR and UV spectra while NMR signals for the methyl proton (τ 8·96, doublet, J = 4 Hz) and no olefinic proton resonances confirmed that the hydrogenation had proceeded to saturate the trisubstituted double bond.

On the other hand prolonged hydrogenation of dihydrothamnosin yielded octahydrothamnosin which exhibited spectral data consistent with structure IX.

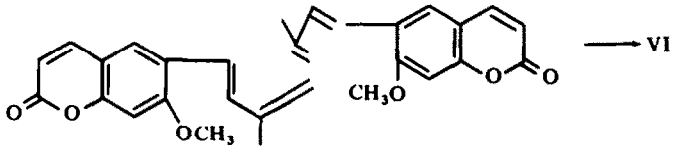


IX

In conclusion, the above epoxidation, ozonization and hydrogenation products of dihydrothamnosin had now completely identified the nature of the trisubstituted double bond in thamnosin and served to confirm the correctness of the structural postulate VI for this natural product.

Thamnosin represents a novel system which until recently had not been previously encountered in any natural source.*

Biogenetically, thamnosin is also an interesting molecule. Its structure suggests that a plausible biosynthetic pathway may include a Diels–Alder type reaction of the appropriately unsaturated monomeric unit as shown below. It is of distinct interest that we have recently isolated a new coumarin, thamnosmin,[1,2] which, in effect has the necessary functionality for conversion to thamnosin. Biosynthetic experiments in this area are presently under investigation.



EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet (UV) spectra were recorded in MeOH on a Cary 14 spectrophotometer, and the infrared (IR) spectra were taken on Perkin–Elmer Model 21 and Model 137 spectrophotometers. Nuclear magnetic resonance (NMR) spectra were recorded in deuteriochloroform (unless otherwise indicated) at 100 MHz on Varian HA100 or at 60 MHz on Varian A60 instruments. The chemical shifts are given in the Tiers τ scale with reference to TMS as the internal standard. Mass spectra were recorded on an A.E.I. MS-9 mass spectrometer. Analyses were performed by Dr. A. Bernhardt, Mulheim (Ruhr), Germany and Mr. P. Borda of the microanalytical laboratory, University of British Columbia. Silica gel G and Woelm neutral alumina containing electronic phosphor were used for thin layer chromatography (TLC).

Thamnosin

The crude thamnosin[3] was recrystallized three times from benzene–CH_2Cl_2 to provide the analytical sample (as prisms), m.p. 244–247°; one bright fluorescent spot on TLC (silica gel G, $CHCl_3$: EtOAc (1:1)). $[\alpha]_D^{20°}$ 0°; IR (KBr): 1725, 1610, 1557 (α-pyrone), 980 (trans-disubstituted double bond), 820 (trisubstituted double bond) cm^{-1}. UV: λ_{max} (ε); 227 (30,000), 256 (23,100), 298 (sh 14,800), 333 mμ (22,900); λ_{min} (ε): 243 (20,600), 282 (12,100). NMR signals (100 MHz): 2·46 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·50 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·89 (1H, singlet, H—C_5 of coumarin), 2·94 (1H, singlet, H—C_5 of coumarin), 3·37 (1H, singlet, H—C_8 of coumarin), 3·39 (1H, singlet, H—C_8 of coumarin), 3·82 (1H, doublet, J = 16 Hz, H_B—C=C), 3·83 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 3·85 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 3·98 (1H, doublet, J = 16 Hz, H_A—C=C), 4·75 (1H, multiplet, H_D—C=C), 6·18 (1H, multiplet, H_C—C), 6·27 (3H, singlet, CH_3O—C_7 of coumarin), 6·29 (3H, singlet, CH_3O—C_7 of coumarin), 8·20 (3H, singlet, CH_3—C=C), 8·78 (3H, singlet, CH_3—C(—)—C=C). Found: C, 74·26; H, 5·74; O, 20·08; O—Me, 12·87. Calc for $C_{30}H_{28}O_6$: C, 74·36; H, 5·82; O, 19·81; (2) O—Me, 12·7%. Molecular ion at m/e 484·188 ($C_{30}H_{28}O_6$ requires 484·189).

* While our initial communication was in press, a publication on the chemical constituents of Australian Zanthoxylum species appeared, G. B. Guise, E. Ritchie, R. G. Senior and W. C. Taylor, *Austral. J. Chem.* **20**, 2429 (1967). A structural assignment to one of the minor constituents, cyclobisuberodiene, was made chiefly on physical evidence. We have now established through the kind co-operation of Professor E. Ritchie that this compound is identical with thamnosin.

Dihydrothamnosin

Thamnosin (238 mg), in absolute THF (40 ml), was hydrogenated over 10% Pd/charcoal (220 mg). The hydrogen uptake ceased after 25 min when 1 mol had been absorbed. After removal of the catalyst and solvent, the product was recrystallized from benzene–light petroleum to give dihydrothamnosin (174 mg), m.p. 226–228°. This compd displayed one dull fluorescent spot on TLC (silica gel, $CHCl_3$: EtOAc (1:1)) whose R_f value was the same as that of thamnosin. IR (KBr): 1728, 1618, 1563 (α-pyrone), 820 (trisubstituted double bond) cm^{-1}. UV: λ_{max} (ε): 224 (36,300), 246 (sh 13,300), 254 (12,000), 300 (sh 14,500), 330 mμ (27,200); λ_{min} (ε): 266 (5900). NMR signals (100 MHz): 2·47 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·53 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·93 (1H, singlet, H—C_5 of coumarin), 3·07 (1H, singlet, H—C_5 of coumarin), 3·28 (1H, singlet, H—C_8 of coumarin), 3·33 (1H, singlet, H—C_8 of coumarin), 3·86 (2H, doublet, J = 9·5 Hz, 2 H—C_3 of coumarin), 4·83 (1H, multiplet, H_D—C=C), 6·33 (1H, doublet, J = 3·5 Hz, H_C—C), 6·22 (3H, singlet, CH_3O—C_7 of coumarin), 6·25 (3H, singlet, CH_3O—C_7 of coumarin), 8·26 (3H, singlet, CH_3—C=C), 8·97 (3H, singlet, CH_3—C). Found: C, 73·47; H, 6·43; O, 20·25; O—Me, 12·87. Calc for $C_{30}H_{30}O_6$: C, 74·07; H, 6·23; O, 19·73; (2) O—Me, 12·8%. Molecular ion at m/e 486·204 (Calc for $C_{30}H_{30}O_6$: 486·204).

Thamnosindiol

Thamnosin (223 mg) was dissolved in absolute THF (40 ml) and OsO_4 (140 mg, 1·2 mol) was added to the soln. The mixture was allowed to stand for 3 days at room temp and then MeOH (100 ml) was added. Dry H_2S was passed through the mixture for 20 min. The sulfide was filtered off to give a pale yellow soln. Evaporation of the solvent gave crystalline thamnosindiol (150 mg). Thamnosindiol crystallized as prisms from MeOH, with one molecule of solvent, (a) m.p. 273–276°. Found: C, 67·99; H, 6·40. Calc for $C_{30}H_{30}O_8 \cdot CH_3OH$: C, 67·61; H, 6·23%. These prisms were ground and dried in the drying pistol for 3 hr at 100° to afford unsolvated thamnosindiol (b), m.p. 243–248°, reforming plates, m.p. 267–272°. Found: C, 69·01; H, 6·33. Calc for $C_{30}H_{30}O_8$: C, 69·49; H, 5·79%. Recrystallization from ethanol afforded the unsolvated thamnosindiol (c) as plates, m.p. 243–248°, reforming plates, m.p. 269–272° mixed melting point with thamnosindiol (b) showed no depression. Found: C, 69·78; H, 5·91; O, 24·51; O—Me, 11·71. Calc for $C_{30}H_{30}O_8$: C, 69·49; H, 5·83; O, 24·68; (2) O—Me, 12·0%. In addition to the above unsolvated plates (c), thamnosindiol crystallized, with one molecule of solvent, as prisms (d), m.p. 267–272°. Found: C, 68·01; H, 6·13; O, 25·72. Calc for $C_{30}H_{30}O_8 \cdot C_2H_5OH$: C, 68·06; H, 6·43; O, 25·51%. These prisms (d) were ground and dried at 100° in the drying pistol for 6 hr to yield unsolvated thamnosindiol (e), m.p. 243–247°, reforming plates, m.p. 266–272°, whose mixed melting point with thamnosindiol (c) showed no depression.

The above thamnosindiol (a, b, c, d and e) showed one spot, respectively, on TLC with the identical R_f values (alumina and silica gel, benzene–EtOAc, $CHCl_3$–EtOAC). IR (KBr): 3480 (hydroxyl), 1725, 1620, 1565 (coumarin), 820 (trisubstituted double bond) cm^{-1}. UV λ_{max} (ε): 223 (36,800), 251 (sh 12,900), 300 (sh 18,200), 328 (29,700), λ_{min} (ε): 267 (6400) mμ. NMR signals (100 MHz): 2·43 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·49 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·61 (1H, singlet, H—C_5 of coumarin), 2·80 (1H, singlet, H—C_5 of coumarin), 3·25 (1H, singlet, H—C_8 of coumarin), 3·49 (1H, singlet, H—C_8 of coumarin), 3·82 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 3·92 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 4·75 (1H, doublet, J = 5 Hz, H_D—C=C), 4·98 (1H, multiplet, H_A—C—O—), 6·16 (3H, singlet, CH_3O—C_7 of coumarin), 6·28 (1H, doublet, J = 5 Hz, H_C—C), 6·47 (3H, singlet, CH_3O—C_7 of coumarin), 6·6 (2H, multiplet, 2HO—), 7·03 (1H, singlet, H_B—C—O—), 7·8 and 8·3 (4H, two sets of doublets, J = 7 Hz, >C—CH_2—CH_2—C), 8·21 (3H, singlet, CH_3—C=C), 8·69 (3H· singlet, CH_3—C). NMR signals (+D_2O, 100 MHz): 4·98 (1H, sharp singlet, H_A—C—OD), 5·30 (singlet, HOD), no peaks at 6·6 (2 DO—), 7·03 (1H, sharp singlet, H_B—C—OD), the rest of the peaks remained the same. High resolution mass determination m/e 500 ($C_{30}H_{30}O_8$—H_2O) + peak: 500·187 (Calc for $C_{30}H_{28}O_7$: 500·184).

Periodate cleavage of thamnosindiol

To a soln of thamnosindiol (200 mg), in MeOH (220 ml), was added HIO_4 aq (1·5 mole) and the reaction mixture was allowed to stand for 24 hr. The solvent was evaporated and the residual material was extracted with CH_2Cl_2. The organic layer was washed with water, $NaHCO_3$ aq and water and dried over Na_2SO_4. Removal of the solvent gave a white solid. The white material was purified by preparative TLC on silica gel ($CHCl_3$: EtOAc (1 : 1)). After the plate was developed, a small portion of the plate was sprayed with 2,4-DNP reagent to give two distinct bands. The more polar compound (aldehyde-I) showed an orange color while the less polar compound (aldehyde-II) was yellow in color. Extraction of these two fractions with MeOH and $CHCl_3$ yielded aldehyde-I (49 mg) and aldehyde-II (25 mg). Aldehyde-I was crystallized as prisms from MeOH, m.p. 242–246°. IR (KBr): 1735, 1670, 1610 (α-pyrone, aldehyde). UV: λ_{max} 255, 308, 329, 342 (sh) mμ, λ_{min} 237, 278, 318 mμ. UV (+ $NaBH_4$): λ_{max} 222, 251 (sh), 295 (sh), 327 mμ, λ_{min} 261 mμ. NMR signals [100 MHz in $(CF_2Cl)_2C(OD)_2$]: −0·23 (1H, singlet, —CHO), 1·90 (1H, singlet, H—C_5 of coumarin), 2·09 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 3·00 (1H, singlet, H—C_8 of coumarin), 3·75 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 6·01 (3H, singlet, CH_3O—C_7 of coumarin). Molecular ion at m/e 204·042 (Calc for $C_{11}H_8O_4$: 204·042).

An authentic sample of 7-methoxycoumarin-6-aldehyde, m.p. 248–251°, was obtained from Dr. F. E. King[10] (King *et al.*[7] give m.p. 252–253°). IR (KBr): 1735, 1670, 1610 cm^{-1}. UV: λ_{max} 255, 308, 328, 342 (sh) mμ, λ_{min} 237, 277, 316 mμ. Mixture melting point with aldehyde-I: m.p. 243–246°.

Aldehyde-I was identical with 7-methoxycoumarin-6-aldehyde by all criteria: mixed m.p.; R_f values on TLC (silica gel and alumina, $CHCl_3$, $CHCl_3$–EtOAc, ETOAc, benzene–EtOAC); superimposable UV and IR spectra.

Aldehyde-II resisted crystallization but data was obtained on TLC pure material. IR ($CHCl_3$): 1720, 1615 cm^{-1} (α-pyrone, saturated aldehyde). UV: λ_{max} 229, 254 (sh), 296 (sh), 328 mμ, λ_{min} 261 mμ. NMR signals (100 MHz): 2·45 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·85 (1H, singlet, H—C_5 of coumarin), 3·31 (1H, singlet, H—C_8 of coumarin), 3·84 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 4·76 (1H, multiplet, H_D—C(—)=C<), 5·84 (1H, doublet, H_C—C<), 6·20 (3H, singlet, CH_3O—C_7 of coumarin), 8·21 (3H, singlet, CH_3—C(—)=C(—)—), 8·82 (3H, singlet, CH_3—C<). Molecular ion at m/e 312·138 (Calc for $C_{19}H_{20}O_4$: 312·136).

Dihydrothamnosinoxide

Dihydrothamnosin (220 mg), in $CHCl_3$ (50 ml), was treated with *m*-chloroperbenzoic acid (1·5 mole) and the soln was maintained at room temp for 36 hr. The soln was then washed with $NaHCO_3$ aq and dried over Na_2SO_4. Removal of the solvent gave crystalline material (200 mg). Recrystallization from diisopropyl ether afforded dihydrothamnosinoxide as plates, m.p. 243–246°. IR (KBr): 1725, 1612, 1565 cm^{-1} (coumarin). UV, λ_{max} (ε): 223 (39,700), 243 (sh 12,800), 253 (sh 9700), 298 (sh 17,300), 329 mμ (27,400), λ_{min} (ε): 263 (3900). NMR signals (100 MHz): 2·47 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·51 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 3·00 (1H, singlet, H—C_5 of coumarin), 3·05 (1H, singlet, H—C_5 of coumarin), 3·26 (1H, singlet, H—C_8 of coumarin), 3·28 (1H, singlet, H—C_8 of coumarin), 3·85 (2H, doublets, J = 9·5 Hz, H—C_3 of coumarin), 6·16 (6H, singlets, 2 CH_3O—C_7 of coumarin), 6·4 (1H, multiplet, H_C—C<), 7·11 (1H, broad singlet, H_D—C(—O—)—C<), 8·59 (CH_3, singlet, CH_3—C(—O—)—C<), 9·17 (3H, singlet, CH_3—C<). Found: C, 72·00; H, 5·77; O, 22·29; O—Me, 12·15. Calc for $C_{30}H_{30}O_7$: C, 71·69; H, 6·02; O, 22·28; (2) O—Me, 12·3%. Molecular ion at m/e 502·202 (Calc for $C_{30}H_{30}O_7$: 502·199).

Attempted epoxide opening on dihydrothamnosinoxide

Dihydrothamnosinoxide (10 mg) was added to a boiling 5% oxalic acid aq. (3 ml) and refluxing continued for 30 min. The soln was cooled and extracted with CH_2Cl_2. The extracts were washed with 5% $NaHCO_3$ aq and dried over Na_2SO_4. The solvent was removed to give a white residue (8 mg). This residue was identified to be the recovered starting oxide by TLC, UV and IR spectra. Under more forcing conditions (for example refluxing in dioxane for 2 hr) the oxide gave intractable mixtures.

Attempted hydroxylation of dihydrothamnosin

Dihydrothamnosin (50 mg), in absolute THF (2 ml), was treated with OsO_4 (31 mg, 1·2 mole). The reac-

tion mixture was allowed to stand at room temp for 5 days, and then was stirred with a soln of $NaHSO_4$ (100 mg) in water (5 ml) and MeOH (10 ml) for 20 hr. The soln was separated, acidified with a few drops of AcOH, concentrated to a small volume and extracted with $CHCl_3$. The organic layer was separated and dried over Na_2SO_4. Evaporation of the solvent afforded a grayish brown solid (23 mg). TLC on silica gel ($CHCl_3$–EtOAc) indicated that the major component in this mixture was recovered starting material. Preparative TLC on silica gel (with very poor recovery) showed that the starting material represented 60% while a few more polar compounds represented the remaining 40% of the reaction mixture.

Controlled ozonation of dihydrothamnosin

Dihydrothamnosin (100 mg), in CH_2Cl_2 (40 ml), was cooled to $-78°$ and a slow stream of ozone was passed through the solution for 60 min until the excess ozone was detected with aqueous KI–boric acid at the outlet. After ozonization, to the soln was added 10% Pd/charcoal (20 mg) and the mixture was shaken under H_2 atmosphere for 10 min. The catalyst was then filtered off and the filtrate evaporated under reduced pressure. The crude product (90 mg) was purified by preparative TLC (silica gel, EtOAc–$CHCl_3$) and the aldehydic band was detected by 2,4-DNP as spray reagent. Extraction of the aldehyde by $CHCl_3$–MeOH afforded an amorphous solid (36 mg) m.p. 135–140°, designated as ketoaldehyde-III. IR ($CHCl_3$): 1721, 1616 cm^{-1} (coumarin, aldehyde, methyl ketone). UV, λ_{max} (ε): 223 (42,900), 254 (sh 12,000), 296 (sh 15,500), 329 mμ (29,900), λ_{min} (ε): 265 mμ (4800). NMR signals (100 MHz): 0·02 (1H, doublet, J = 2 Hz, —CHO), 2·39 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·44 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·68 (1H, singlet, H—C_5 of coumarin), 2·86 (1H, singlet, H—C_5 of coumarin), 3·18 (1H, singlet, H—C_8 of coumarin), 3·27 (1H, singlet, H—C_8 of coumarin), 3·77 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 3·81 (1H, doublet J = 9·5 Hz, H—C_3 of coumarin), 5·76 (1H, doublet, J = 2 Hz, $\underline{H}_C$—$\overset{|}{\underset{|}{C}}$—CHO), 6·15 (6H, singlets, 2 CH_3O—C_7 of coumarin), 7·87 (3H, singlet, CH_3—CO—), 8·91 (3H, singlet, CH_3—$C\langle$). High resolution mass determination, m/e 500 $(C_{30}H_{30}O_8-H_2O)^+$ peak: 500·184 (Calc for $C_{30}H_{28}O_7$: 500·184).

$NaBH_4$ *Reduction of ketoaldehyde-III*

Ketoaldehyde-III (16 mg), in isopropanol (2 ml) and $CHCl_3$ (1 ml), was reduced with $NaBH_4$ (8 mg). After the mixture was allowed to stand at room temp for 55 min the solvent was removed *in vacuo*. The resulting residue was extracted with $CHCl_3$. Evaporation of the $CHCl_3$ gave an amorphous solid (8 mg). IR ($CHCl_3$): 3436 (hydroxyl), 1718, 1613, 1560 (coumarin) cm^{-1}. UV: λ_{max} 225, 254, 298 (sh), 329 mμ. NMR signals (100 MHz): 3·43 (2H, doublet, J = 9·5 Hz, 2H—C_4 of coumarin), 3·66 (1H, singlet, H—C_5 of coumarin), 3·84 (1H, singlet, H—C_5 of coumarin), 3·29 (2H, singlet, 2H—C_8 of coumarin), 3·85 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 6·15 (6H, singlet, 2 CH_3O—C_7), 5·8–6·4 (6H, multiplet), 8·82 (3H, multiplet, $C\underline{H}_3$—$\overset{|}{C}$H—OH), 9·09 (3H, singlet, CH_3—$C\langle$).

Suberosin

Demethylsuberosin[10] was methylated with MeI and K_2CO_3 by a usual method. Recrystallization from diisopropyl ether gave suberosin (7-methoxy-6-isopent-2′-enylcoumarin) as prisms, m.p. 82–87° (King et al[7] give m.p. 87–88°). IR ($CHCl_3$): 1724, 1621, 1563 (coumarin, α-pyrone) cm^{-1}. UV: λ_{max} (ε): 223 (22,800), 253 (3410), 297 (sh 6880), 330 (15,400) mμ. NMR signals (100 MHz): 2·47 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·90 (1H, singlet, H—C_5 of coumarin), 3·30 (1H, singlet, H—C_8 of coumarin), 3·87 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 4·78 (1H, broad triplet, J = 7 Hz, H—$C_{2'}$), 6·18 (3H, singlet, CH_3O—C_7 of coumarin), 6·74 (2H, doublet, J = 7 Hz, 2 H—$C_{1'}$), 8·27 (3H, singlet CH_3—$\overset{|}{\underset{|}{C}}$=$\overset{|}{\underset{|}{C}}$—), 8·33 (3H, singlet, CH_3—$\overset{|}{\underset{|}{C}}$=$\overset{|}{\underset{|}{C}}$=).

Tetrahydrothamnosin

Dihydrothamnosin (50 mg), in AcOH (25 ml), was hydrogenated over 10% Pd/charcoal (100 mg). The hydrogenation was interrupted when 1 mole of H_2 was absorbed. The catalyst was filtered off and removal of the solvent gave an amorphous solid (40 mg), tetrahydrothamnosin. IR ($CHCl_3$): 1721, 1623, 1560 (coumarin) cm^{-1}. UV, λ_{max} end absorption (220 mμ), 254 (sh), 300 (sh), 332 mμ, λ_{min} 266 mμ. NMR signals

(100 MHz): 2·46 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·50 (1H, doublet, J = 9·5 Hz, H—C_4 of coumarin), 2·92 (1H, singlet, H—C_5 of coumarin), 2·97 (1H, singlet, H—C_5 of coumarin), 3·26 (2H, singlets, 2 H—C_8 of coumarin), 3·82 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 3·86 (1H, doublet, J = 9·5 Hz, H—C_3 of coumarin), 6·16 (3H, singlet, CH_3O—C_7 of coumarin), 6·18 (3H, singlet, CH_3O—C_7 of coumarin), 8·96 (3H, doublet, J = 4 Hz, CH_3—CH—), 9·16 (3H, singlet, CH_3—C<).

octahydrothamnosin

Dihydrothamnosin (49 mg), in CH_2Cl_2–MeOH (20 ml, 1:1) was hydrogenated over 10% Pd/charcoal. The H_2 uptake ceased after 3 mole and the catalyst was filtered off. Removal of the solvent gave an amorphous solid (46 mg), octahydrothamnosin. IR ($CHCl_3$): 1761, 1618 cm^{-1} (C=O, aromatic). UV, λ_{max} end absorption (220 mμ), 285 mμ, λ_{min} 253 mμ. NMR signals (60 MHz): 3·25 (2H, broad singlets, 2 H—C_5), 3·55 (2H, broad singlets, 2 H—C_8), 6·28 (3H, singlet, CH_3O—C_7), 6·32 (3H, singlet, CH_3O—C_7), 9·08 (3H, multiplet, CH_3—CH), 9·23 (3H, singlet, CH_3—C<).

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