Proc. Indian Acad. Sci., Vol. 87 A (Chemical Sciences-3), No. 6, June 1978, pp. 173–179,

Chemical constituents of *Atalantia racemosa* Wt. and Arn. Structure and synthesis of racemosin, a novel pyranocoumarin*

BALAWANT S JOSHI, DILIP H GAWAD and K R RAVINDRANATH
CIBA-GEIGY Research Centre, Goregaon, Bombay 400 063

MS received 18 January 1978; revised 2 March 1978

Abstract. Phytochemical investigation of the aerial parts of *Atalantia racemosa* Wt. and Arn. gave the known compounds xanthyletin, xanthotoxin and friedelin. The structure (I) for the new coumarin, racemosin, has been deduced on the basis of UV, IR, NOE, proton and carbon-13 NMR spectral data. The assigned structure for the natural product has been confirmed by synthesis.

Keywords. Structure determination; synthesis; ^1H– and ^{13}C-NMR spectra; NOE; rutaceae.

1. Introduction

Atalantia racemosa Wt. and Arn. (Family: Rutaceae) is a medium-sized tree growing in most of the forest regions of the Western Ghats. Antiviral activity has been observed in *Ranikhet disease* virus in the crude extracts of the plant excluding the roots (Bhakuni *et al* 1971). No other work appears to have been reported on this plant.

We wish to report in this paper the phytochemical investigation on the aerial parts of the plant.

2. Results and discussion

2.1. *Structure of racemosin*

Hexane extracts of the stem and leaves on concentration and cooling gave a colourless crystalline compound $C_{16}H_{16}O_5$, m.p. 125–126° designated as racemosin. UV spectrum showed features typical of the pyranocoumarin ring system (Lassak and Pinhey 1969) showing peaks at 224, 267, 272 and 328 nm. The IR spectrum had two high intensity carbonyl bands at 1710 and 1600 cm^{-1} due to an α, β-unsaturated lactone. Mass spectrum of racemosin gave the molecular ion at M^+ 288, the base peak at m/e 273 by loss of $Me^\bullet$, and the corresponding doubly charged fragment at m/e 136·5 characteristic of 2,2-dimethyl chromenes. The ion at m/e 258 arises from m/e 273 probably by loss of methyl radical from the methoxyl group (Barnes and Occolowitz 1964).

*Contribution No. 509 from the CIBA-GEIGY Research Centre.

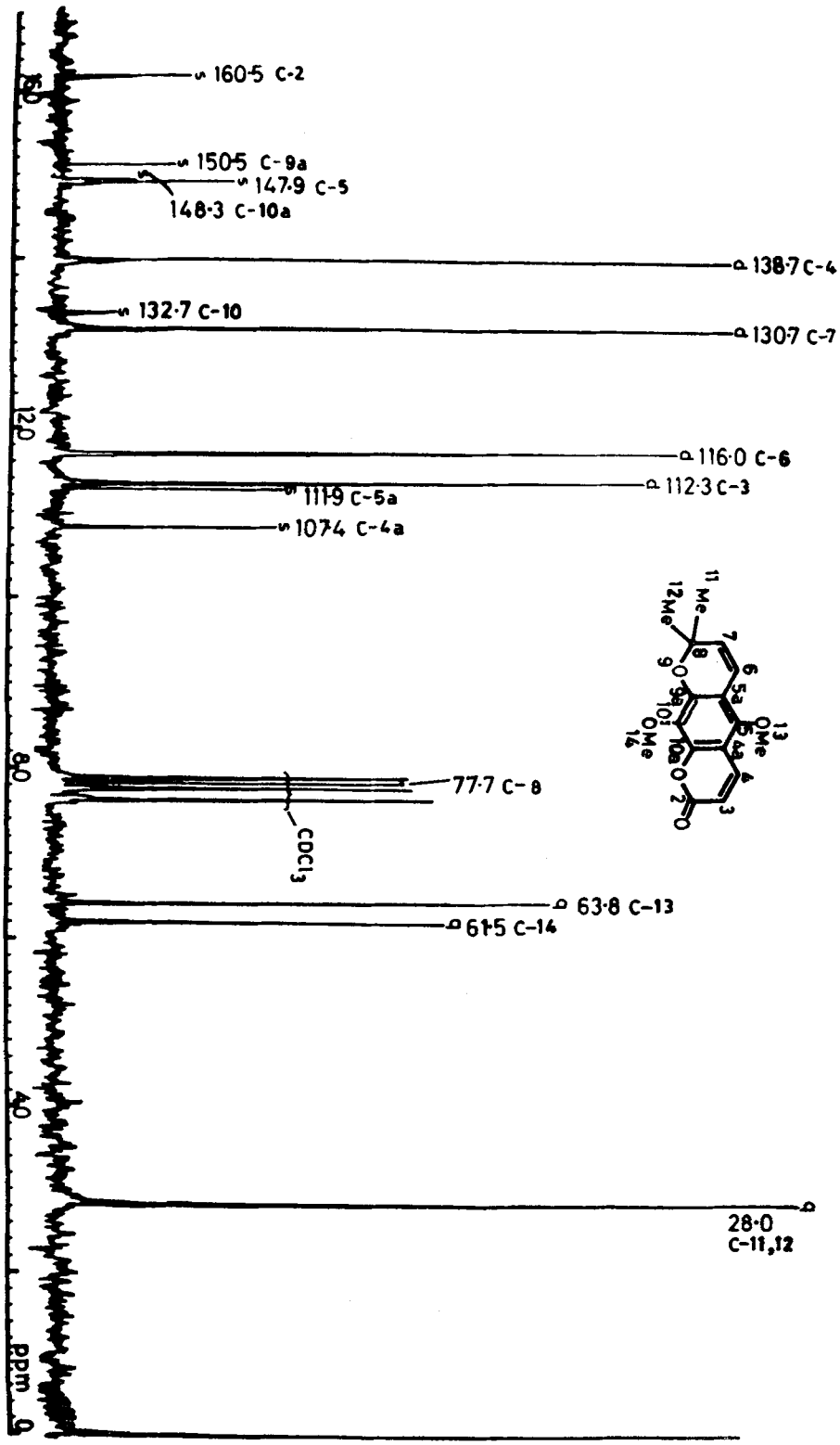


Figure 1. ^{13}C-NMR spectrum of racemosin (I) in $CDCl_3$ (proton noise decoupled).

The 100 MHz PMR spectrum readily resolved all the sixteen protons of racemosin. An AB quartet (J=10 Hz) δ7·75 (1H) and 6·13 (1H) confirmed the presence of an α,β-unsaturated ring system indicating the C-4 and C-3 protons of the coumarin nucleus (Neilsen 1970). The presence of a 2,2-dimethyl chromene ring system encountered in pyranocoumarins was suggested by the occurrence of a singlet for *gem*-dimethyl groups at δ1·44 (6H) and an AB quartet (J=10 Hz) of the chromene centered at δ6·49 (H-6) and 5·65 (H-7) (Arthur and Ollis 1963; Tomimatsu *et al* 1969). The presence of two methoxyl groups was evident from the singlets appearing at δ3·85 (3H) and 3·76 (3H).

The above data indicate six possible isomeric structures (I–VI) which should be considered for racemosin. Since all these structures are oxygenated in the 5- or 7-positions as in most of the naturally occurring coumarins (Davon and Scott 1975), it was difficult to eliminate some of the possibilities on biogenetic considerations. An attempt was made to choose a structure for racemosin by measurement of the intramolecular nuclear Overhauser effect (NOE) involving the methoxy group and the olefinic protons. The observed NOE is shown in table 1. Three structures (I) (II) and (IV) are possible wherein one of the methoxyl groups is in close proximity to both olefinic protons (Fuhrer *et al* 1970; Tomimatsu *et al* 1972). On saturation of the OMe signals, the intensities of both olefinic H-4 and H-6 in racemosin were appreciably increased. The NOE results exclude structure (VI) where no olefinic proton is close to either of the methoxyl groups and also structures (III) and (V) where only one of the olefinic proton will be affected.

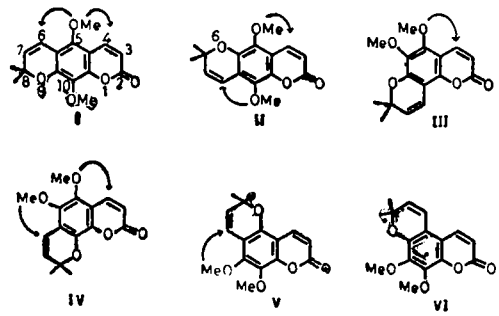
A unique choice for the structure was made on the basis of the ^{13}C-NMR spectrum (figure 1).

The proton decoupled ^{13}C-NMR spectrum of racemosin (figure 1) exhibits 15 lines with the lowest frequency signal corresponding to twice the intensity of the other proton-bearing carbon atoms. The off resonance decoupled spectrum also revealed the presence of eight singlets, four doublets and three quartets. Of special significance is the downfield shifts of C-5 (147·9), C-9a (150·5) and C-10 (132·7) due to the presence of oxygen on these carbons, from the normal values of 127·4 (C-5), 131·2 (C-9a) and 115·9 (C-10) ppm in coumarin. Also, C-5a shifts as expected, upfield from 123·9 to 111·9 ppm (Wenkert *et al* 1976).

The structure (I) carries on oxygen on C-9a whereas in (II) and (IV) the chromene and methoxyl oxygen is on C-5a. It has been established that the coumarin carbon C-3 shows a shielding of about 4·0 Hz only when C-9a carries an oxygen function (Wenkert *et al* 1976; Cussans and Huckerby 1975). In racemosin, the C-3 signal appears at 112·3 as compared to 116·1 in coumarin. Thus the ^{13}C-NMR spectrum is in conformity with the formulation (I) for racemosin.

Table 1. Observed nuclear overhauser effects for racemosin on saturation of the methoxyl signals at δ 3·8

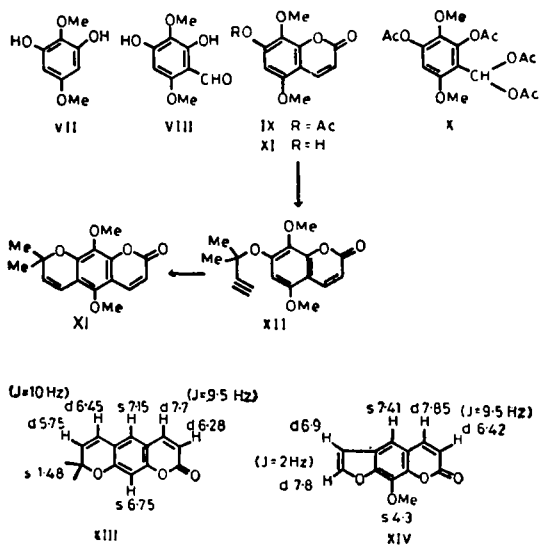
Increase in integrated intensities of signals of			
H-3	H-4	H-6	H-7
Nil	9%	13%	Nil



2.2. *Synthesis of racemosin*

Since the spectral data indicated structure (I) for the natural product it was desirable to confirm this by an unambiguous synthesis.

2,5-Dimethoxyresorcinol (VII) (Clarke and Robertson 1949) under Gatterman reaction conditions gave the aldehyde (VIII), which was converted to 7-acetoxy-5,8-dimethoxy coumarin (IX). When the acetylation was carried out for a shorter period, the acetoxy hemiacetal (X) was obtained. Hydrolysis of (IX) afforded the phenol (XI), which was converted to the 1,1-dimethylpropargyl ether (XII) (Hlubucek *et al* 1969) by refluxing with 3-chloro-3-methylbut-1-yne. Rearrangement of the ether (XII) was effected in boiling diethylaniline to afford (I) identical in all respects with racemosin.



2.3. *Isolation of other constituents*

After separation of racemosin. the hexane extract was chromatographed on silica gel. The first fraction gave a triterpene $C_{30}H_{50}O$. m.p. 259–260° identified as friedelin. The second fraction afforded a coumarin $C_{14}H_{12}O_3$, m.p. 130–131° which was readily characterized from its NMR and mass-spectra as xanthyletin (XIII). The third eluted fraction also gave a coumarin $C_{12}H_8O_4$, m.p. 145–146° identical with xanthotoxin (XIV) (Davon and Scott 1975).

3. Experimental

All melting points are uncorrected. UV and IR spectra were determined on Beckmann DK-2A and Perkin-Elmer infracord or Model 421 spectrophotometers. NMR spectra were taken on Varian A-60 and XL-100 spectrophotometers in $CDCl_3$ solution with TMS as internal reference standard. The ^{13}C-NMR spectra were measured on a Varian XL-100 spectrometer at 25·2 MHz in the pulsed mode. Chemical shifts δ are given in ppm downfield from Me_4Si. Mass spectra were determined on Atlas CH-7 instrument. Known substances were identified by mixed m.p. determination and comparisons of IR spectra with authentic samples.

3.1. *Extraction of Atalantia racemosa* Wt. & Arn.

The dried and milled aerial parts of the plant collected in Mahabaleshwar (3·5 kg were extracted with hexane (2×15 l) and the extract concentrated under vacuum to 100 ml. The solid separated was collected (400 mg) and crystallized twice from methylene chloride-hexane to afford colourless plates of racemosin (I) (250 mg) m.p. 125–126° (TLC, si gel, chloroform, R_f 0·6). UV λ_{max} (ethanol) 224, 228, 267, 272 and 328 nm (log ϵ, 4·33, 4·31, 4·44, 4·46 and 4·06). IRν_{max} (KBr) 2980, 2940, 1710, 1600, 1585, 1455, 1430, 1390, 1375, 1360, 1335, 1300, 1280, 1235, 1210, 1195, 1185 1165, 1135, 1120, 1050, 985, 950, 830, 815 and 750 cm^{-1}. MS m/e 288 (M^+, 25%), 273 (100), 258 (15), 243 (30), 225 (15), 215 (20), 197 (15), 144 (30), 136·5 (30), 129 (70), 115 (80). (Found: C, 66·7; H, 5·9. $C_{16}H_{16}O_5$ requires: C, 66·7; H, 5·6%).

The hexane extract after separation of racemosin was evaporated under vacuum to give a brown coloured oil (15 g). It was dissolved in hexane-benzene (1 : 1) and chromatographed over silica gel (150 g) and gradient eluted with hexane, hexane-benzene, benzene-chloroform and chloroform-methanol. Fractions (150 ml) were collected and the chromatographic separation monitored by TLC.

Fractions 28–29 (elution with benzene) gave on crystallization from hexane-chloroform, colourless needles (5 mg), m.p. 259–260° identical with friedelin.

Fractions 31–35 (elution with benzene) gave on crystallization from hexane-chloroform shining plates (500 mg), m.p. 130–131° identical with xanthyletin.

Fractions 51–53 (elution with benzene: chloroform 1 : 3) afforded on crystallization from hexane-chloroform, colourless needles (70 mg), m.p. 145–146° identical with xanthotoxin.

3.2. 2,4-*Dihydroxy*-3,6-*dimethoxybenzaldehyde* (VIII)

To an ice-cold solution of 2,5-dimethoxyresorcinol (VII) (500 mg) in dry ether (10 ml), was added zinc cyanide (500 mg) and dry hydrogen chloride gas was passed through the mixture at 0°. The mixture was left at room temperature for 24 hr, and the supernatent ether was decanted. The residual solid was washed with dry ether, dissolved in water (10 ml), and the pH adjusted to 5 with sodium carbonate. The solution was heated at 70° for 30 min, the precipitate collected, washed with water and crystallized from methanol to afford yellow needles (400 mg), m.p. 203°. IR, ν_{max} (KBr), 1640, 1600 cm^{-1}. NMR ($CDCl_3$+CD_3SOCD_3): δ 12·5 (s, 1H, OH, chelated, exchanges with D_2O), 10·6 (br, 1H, OH, exchanges with D_2O), 10·0 (s, 1H, CHO), 6·05 (s, 1H,

5-H), 3·8 (s, 3H, OMe), 3.7 (s, 3H, OMe). MS m/e 198 (M^+, 50%), 183 (40), 155 (100). (Found: C, 54·7; H, 5·3. Calc. for $C_9H_{10}O_5$: C, 54·5: H, 5·3%).

3.3. *7-Acetoxy-5,8-dimethoxycoumarin* (IX)

An intimate mixture of 2,4-dihydroxy-3,6-dimethoxy benzaldehyde (3 g), freshly fused sodium acetate (5 g) and acetic anhydride (18 ml) was heated at 175–180° for 24 hr. It was poured over ice, kept at room temperature for 16 hr and filtered. The residue was dissolved in chloroform, washed with 10% sodium bicarbonate (100 ml), water and the organic layer dried over Na_2SO_4 and the solvent evaporated. Crystallization of residue from methylene chloride-methanol gave yellow needles of the coumarin (IX; 1·8 g), m.p. 185-187°. UV λ_{max} (ethanol) 253 and 302 nm (log ϵ, 3·9 and 4·07). IR ν_{max} (KBr) 1760, 1720, 1600 cm^{-1}. NMR ($CDCl_3$): δ 8·0 (d, J=10 Hz, 1H, H-4), 6·45 (s, 1H, H-6), 6·27 (d, J=10Hz, 1H, H-3), 3·95 (s, 3H, OMe), 3·9 (s, 3H, OMe), 2·37 (s, 3H, OAc). MS m/e 264 (M^+, 10%), 222 (90), 207 (100). (Found: C, 59·3; H, 4·9. $C_{13}H_{12}O_6$ requires: C, 59·1; H, 4·6%).

3.4. *2-Bisacetoxymethyl-3,5-diacetoxy-1,4-quinoldimethylether* (X)

A mixture of (VIII; 3g), fused sodium acetate (5 g) and acetic anhydride (18 ml) was heated at 170° for 3 hr, when a solid had separated. It was poured into water, filtered, washed and crystallized from ethanol to afford needles of (X; 2·7 g), m.p. 134–135°. NMR ($CDCl_3$): δ 8·1 (s, 1H, 2-C<u>H</u>), 6·59 (s, 1H, H-6), 3·83 (s, 3H, OMe), 3·75 (s, 3H, OMe), 2·37 (s, 3H, 3 or 5-OAc), 2·31 (s, 3H, 3 or 5-OAc), 2·05 (s, 6H, CH-<u>OAc_2</u>). (Found: C, 53·7; H, 5·6. $C_{17}H_{20}O_{10}$ requires: C, 53·1; H, 5·3%).

3.5. *5,8-Dimethoxy-7-hydroxycoumarin* (XI)

7-Acetoxy-5,8-dimethoxycoumarin (0·5 g), ethanol (5 ml) and 6% sodium hydroxide (1·5 ml) were heated at 70° for 15 min. Water (6 ml) was added and the solution heated for 10 min, cooled and acidified with 35% HCl. The precipitate was collected, washed free of acid and dried. The crude phenol was purified by chromatographic separation over silica gel using benzene as solvent and eluent. Crystallization from methylene chloride hexane afforded fine needles (XI; 250 mg), m.p. 195–196°. UV λ_{max} (ethanol) 260, 330 nm (log ϵ 3·96 and 4·1) . IR, ν_{max} (KBr), 1700,1600, 1570 cm^{-1}., NMR (CD_3SOCD_3): δ7·95 (d, J=9·5Hz, 1H, H-4), 6·45 (s, 1H, H-6), 6·1 (d, J=9·5 H_2) 1H, H-3), 3·9 (s, 3H, OMe), 3·83 (s, 3H, OMe), 3·5 (br, 1H, OH, exchanged with D_2O). MS m/e 222 (M^+, 50%) 207 (100), 179 (30), 157 (20). (Found: C, 59·5; H, 4·7. $C_{11}H_{10}O_5$ requires: C, 59·5; H,, 4·5%).

3.6. *5,8-Dimethoxy-7-(1,1-dimethylpropargyloxy) coumarin* (XII)

A mixture of 5,8-dimethoxy-7-hydroxy coumarin (XI; 300 mg), potassium iodide (125 mg), anhydrous potassium carbonate (1·5 g), 3-chloro-3-methylbut-1-yne (0·8 g) in dry acetone (15 ml) was refluxed for 48 hr. The mixture was filtered and the aectone was removed under vacuum. The yellow gum (300 mg) was dissolved in benzene and chromatographed over silica gel (5 g), eluting with hexane: benzene (1 : 1).

Fractions (50 ml) each 5 to 18 gave a solid which on crystallization from methanol afforded yellow needles (XII; 125 mg), m.p. 156°. UV λ_{max} 257 and 315 nm (log ϵ, 3·98 and 4·11). IR (KBr), ν_{max} 3300, 1700, 1600 cm^{-1}. NMR ($CDCl_3$): δ 7·95 (d, J=9·5 Hz, 1H, H-4), 7·05 (s, 1H, H-6), 6·2 (d, J=9·5 Hz, 1H, H-3), 3·9 (s, 6H, OMe), 2·72 (s, 1H, ≡C—H), 1·73 (s, 6H, $\diagup\!\!\diagdown^{Me}_{Me}$). MS, m/e 288 ($M^+$, 5%), 273 (5), 257 (10), 222 (100), 207 (95), 179 (50). (Found: C, 66·6; H, 5·5. $C_{16}H_{16}O_5$ requires: C, 66·7; H, 5.6%).

3.7. 5,10-*Dimethoxy*-8,8-*dimethyl*-2-*oxo* 2*H*,8*H benzo* [1, 2–b: 5, 4–b']-dipyran (I), (*racemosin*)

The coumarin (XII; 50 mg) in distilled diethyl-aniline (0·5 ml) was heated at 225° in an atm. of N_2 for 2 hr. The brown liquid was taken up in methylene chloride and washed with 15% HCl, water and the organic layer dried over Na_2SO_4. Removal of the solvent gave a gum (50 mg), which was dissolved in benzene and chromatographed over silica gel (4 g). It was gradient eluted with hexane-benzene, benzene and fractions (25 ml) were monitored by TLC. Fractions 50-56 (eluted with benzene) afforded on crystallization from methanol, colourless plates (4 mg), m.p. 123–125° identical with a sample of racemosin in all respects.

Acknowledgements

The authors wish to thank Dr H Fuhrer, CIBA-GEIGY Ltd., Basle for the 100 MHz PMR and ^{13}C-NMR spectra, Dr S Selvavinayakam and his associates for the analytical, spectral data and Dr R S Grewal for his interest.

References

Arthur H R and Ollis W D 1963 *J. Chem. Soc.* 3910
Barnes C S and Occolowitz J L 1964 *Aust. J. Chem.* **17** 975
Bhakuni D S *et al* 1971 *Indian J. Exptl. Biol.* **9** 93
Clarke J R and Robertson A 1949 *J. Chem. Soc.* 302
Cussans N J and Huckerby T N 1975 *Tetrahedron* **31** 2719
Davon T K and Scott A I 1975 *Handbook of Naturally Occurring Compounds* (New York: Academic Press) Vol. 1
Fuhrer H, Govindachari T R, Joshi B S and Pai B R 1970 *Indian J. Chem.* **8** 198
Hlubucek J, Ritchie E and Taylor W C 1969 *Tetrahedron Lett.* 1369
Lassak E V and Pinhey J T 1969 *Aust. J. Chem.* **22** 2175
Neilsen B E 1970 *Dan. Tidsskr. Farm.* **44** 161
Tomimatsu T, Hashimoto M, Shingu T and Tori K 1969 *J. Chem. Soc. Chem. Commun.* 168
Tomimatsu T, Hashimoto M, Shingu T and Tori K 1972 *Tetrahedron* **28** 2003
Wenkert E *et al* 1976 Topics in Carbon-13 NMR Spectroscopy Vol. 2 ed. G C Levy (New York: John Wiley) Vol. 2 p. 114