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COUMARINS FROM *BROSIMUM RUBESCENS**

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Key Word Index—*Brosimum rubescens*; Moraceae; 7-demethylsuberosin; xanthyletin; luvangetin; brosiparin; brosiprenin; *O*-prenylbrosiparin; 8-hydroxy-7-methoxy-6-prenylcoumarin; 8-hydroxy-7-methoxy-5,6-diprenylcoumarin.

Abstract—The heartwood of *Brosimum rubescens* Taubert contains the 7-hydroxycoumarins 7-demethylsuberosin (IIa) and xanthyletin (Ia) besides the 7,8-dihydroxycoumarins brosiparin (IIb), brosiprenin (IVa) and luvangetin (Ic).

Brosimum rubescens Taubert (= *B. paraense* Huber)[2] is an arboreous Moraceae species. Fairly common in Amazonia, it is known as 'muirapiranga' in Brazil and as 'palo de sangre' in Peru. Its reddish heartwood, which is used in carpentry, contains a surprisingly large proportion of xanthyletin (Ia),[3,4] a coumarin which had previously been mistaken for a steroid.[5] The presence of minor amounts of other compounds, one of them tentatively identified with xanthoxyletin (Ib), has also been reported.[4] Following the suggestion of Dr. K. Brown, Jr., we re-examined the extractives of the heartwood, isolating, besides xanthyletin (Ia), four compounds.

The analytical data of one of these compounds were identical with the data published for 7-demethylsuberosin (IIa), a constituent of *Chloroxylon swietenia*.[6] Indeed, treatment with acid produced dihydroxanthyletin (IIIa), confirming the identification.

IR, UV, PMR and MS data revealed the second compound as a novel coumarin, brosiparin, substituted by a hydroxy, a methoxy and a prenyl group in the aromatic ring. The placement of these groups, as indicated in IIb, involved consideration of the following experimental evidence: (1) the UV spectrum of brosiparin acetate (IIc) showed maxima at 295 and 320 nm, typical of 7-alkoxycoumarins;[7,8] (2) The Gibbs test, which is applicable

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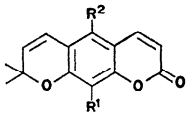
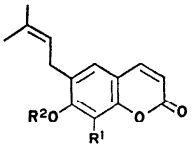
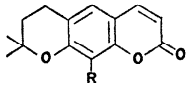
to coumarins,[9] was positive, suggesting the *para*-relationship of the hydroxyl and the unsubstituted aromatic position: (3) Treatment of brosiparin with trifluoroacetic acid led to a trifluoroacetate, precluding the vicinality of the hydroxyl and the prenyl groups. Acid catalyzed cyclization of the prenyl group of brosiparin occurred only after cleavage of the methoxyl with HI. The reaction product was identified, by direct comparison, with 5′,6′-dihydro-8-hydroxy-6′,6′-dimethylpyrano-(2′,3′:7,6)-coumarin (IIIb), obtained by the BF_3 catalyzed condensation[10] of daphnetin[11] and 2-methyl-3-buten-2-ol.



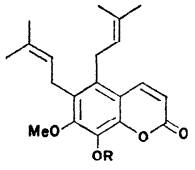
(Ia) $R^1 = H; R^2 = H$
(Ib) $R^1 = H; R^2 = OMe$
(Ic) $R^1 = OMe; R^2 = H$



(IIa) $R^1 = H; R^2 = H$
(IIb) $R^1 = OH; R^2 = Me$
(IIc) $R^1 = OAc; R^2 = Me$
(IId) $R^1 = OPre; R^2 = Me$



(IIIa) R = H
(IIIb) R = OH
(IIIc) R = OMe



(IVa) R = H
(IVb) R = Ac

The analytical data of the third compound were identical with the data published for luvangetin (Ic), a constituent of *Luvanga scandens* Ham.,[12,13] with one exception; the dihydro-derivative had m.p. 99°, while the literature,[12,14] records for dihydroluvangetin (IIIc) the m.p. 131°. The compound, however, is either dimorphous, or this figure must be considered in error. Synthetic dihydroluvangetin, obtained by methylation of IIIb, was found identical with the dihydroderivative of our isolate in all respects, including m.p.

Spectral data revealed the fourth compound as a novel coumarin, brosiprenin, substituted by a hydroxy, a methoxy and two prenyl groups in the aromatic ring. The position of the oxygen functions in brosiparin (IIb) and brosiprenin must be identical; their UV spectra were practically superimposable, in neutral as well as in alkaline medium. Besides, the UV spectrum of brosiprenin acetate was again typical of 7-alkoxycoumarins, while the IR spectrum showed a carbonyl band at 1722 cm^{-1} which is incompatible with a 7-hydroxycoumarin.[15] Brosiprenin should thus have structure IVa, and this was confirmed by comparison with a synthetic specimen, prepared by the Claisen rearrangement of *O*-prenylbrosiparin (IId).

Claisen type rearrangements have lately been shown to occur in nature.[16] and it is conceivable that the biosynthesis of brosiprenin (IVa) might involve such a process. So far, however, we were unable to detect *O*-prenylbrosiparin (IId), the putative precursor of brosiprenin (IVa), in the original extract, in spite of its reasonable stability under the conditions used for solvent extraction of the plant material and chromatography of the extract.

The coumarins of *Brosimum rubescens* belong to two series according to oxygenation either at the 7-position (Ia, IIa) or the 7,8-positions (Ic, IIb, IVa). It is consequently not surprising that the present work failed to confirm the presence of xanthoxyletin (Ib).[4]

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EXPERIMENTAL

The m.ps were taken on a Kofler hot stage microscope and are uncorrected. Merck's Kieselgel 0·05–0·20 mm and PF_{254} were used respectively for column and thin or thick layer chromatography. NMR spectra were registered on a R-10 Perkin–Elmer instrument at 60 MHz. *s*–singlet, *d*–doublet, *t*–triplet, *m*–multiplet. MS were taken on an AEI MS9 instrument. Direct comparison of compounds for identification purposes involved besides m.m.ps also IR, UV and NMR spectra.

Isolation of coumarins from Brosimun rubescens. The powdered heartwood (6·2 kg) was extracted with benzene. The extract gave, after a series of fractional crystallizations from benzene, crystals of Ia (600 g). The mother liquors were united and evaporated. The residue was freed from coloured matter by filtration of its MeOH solution through Sephadex LH-20. The MeOH was evaporated and the residue (9 g) was chromatographed on silica. Elution with $CHCl_3$, gave in order Ia (1 g), sitosterol (40 mg), Ic (200 mg), IVa (500 mg), IIb (500 mg) and IIa (600 mg).

Xanthyletin (Ia). Elongated flat prisms, m.p. and m.m.p. with an authentic sample[4] 130–132° (MeOH). NMR and MS data as required by lit.[17] *Dihydroxanthyletin* (IIIa). Hydrogenation (10% Pd/C, EtOH–AcOH, 1:1) of Ia gave IIIa, prisms, m.p. 122–124° (hexane) [lit.[6] m.p. 122–124°]. IV, UV and NMR data as required by lit.[18]

7-*Demethylsuberosin* (IIa). Long yellow needles, m.p. 133–134° (hexane–acetone) [lit.[6] m.p. 133·5–134°]. ν_{max}^{KBr} (cm^{-1}): 1681, 1613, 1565, 1387, 1130. UV data as required by lit.[6] NMR ($CDCl_3$, τ): 2·33 (*d*, *J* 10·3 Hz, H-4), 2·81 (*s* H-5), 2·94 (*s*, H-8), 3·83 (*d*, *J* 10·3 Hz, H-3), 4·75 (*t*, *J* 6·8 Hz, =C$\underline{H}$), 6·70 (*d*, *J* 6·8 Hz, C$\underline{H}_2$), 8·25 (broad *s*, two C$\underline{H}_3$). MS: M 230 (40%), *m/e* (%) 215 (15), 176 (10), 175 (100), 147 (8). *Dihydroxanthyletin* (IIIa). A soln of IIa (40 mg) in $CHCl_3$ (0·3 ml), treated with 1 drop of $F_3C.CO_2H$, kept at 0° for 24 hr, washed with H_2O, dried and evaporated, yielded a residue which was recrystallized from hexane giving crystals, m.p. and m.m.p. with (IIIa) 122–124°.

Brosiparin (IIb). Yellow crystals, m.p. 121–122° (hexane–acetone). M found: 260·1049; $C_{15}H_{16}O_4$ requires: 260·1048. λ_{max}^{EtOH} (nm): 257, 310 (log ϵ 4·01, 4·14); $\lambda_{max}^{EtOH+NaOH}$ (nm): 280, 327 (log ϵ 4·27, 3·99). ν_{max}^{KBr} (cm^{-1}): 3509, 1725, 1601, 1570, 1445, 1270, 1260, 1150. NMR ($CDCl_3$, τ): 2·30 (*d*, *J* 9·9 Hz, H-4) 3·12 (*s*, H-5), 3·65 (*d*, *J* 9·9 Hz, H-3), 4·70 (*t*, *J* 7·7 Hz, =C$\underline{H}$), 6·00 (*s*, OC$\underline{H}_3$), 6·60 (*d*, *J* 7·7 Hz, C$\underline{H}_2$), 8·20 (*s*, two C$\underline{H}_3$). MS: M 260 (100%), *m/e* (%) 245 (46), 229 (10), 205 (22), 185 (11). *Acetate* (IIc), crystals, m.p. 108–110° (hexane–acetone). λ_{max}^{EtOH} (nm): 292, 320 (log ϵ 4·08, 3·99). ν_{max}^{KBr} (cm^{-1}): 1750, 1725, 1610, 1560, 1200. NMR ($CDCl_3$, τ): 2·30 (*d*, *J* 9·7 Hz, H-4), 2·76 (*s*, H-5), 3·65 (*d*, *J* 9·7 Hz, H-3), 4·60 (*t*, *J* 6·8 Hz, =C$\underline{H}$), 6·08 (*s*, OC$\underline{H}_3$), 6·60 (*d*, *J* 6·8 Hz, C$\underline{H}_2$), 7·54 (*s*, OCOC$\underline{H}_3$), 8·20 (*s*, C$\underline{H}_3$), 8·22 (*s*, C$\underline{H}_3$).

Trifluoroacetic acid addition product. To a solution of brosiparin (40 mg) in $CDCl_3$ (0·3 ml) 1 drop of $F_3C.CO_2H$ was added. The reaction was monitored by NMR spectrometry to its completion after 2 hr at 36°. NMR ($CDCl_3$–$F_3C.CO_2H$, τ): 2·05 (*d*, *J* 9·7 Hz, H-4), 2·98 (*s*, H-5), 3·48 (*d*, *J* 9·7 Hz, H-3), 5·90 (*s*, OC$\underline{H}_3$), 7·20 (*m*, C$\underline{H}_2$), 7·90 (*m*, C$\underline{H}_2$), 8·35 (*s*, two C$\underline{H}_3$). The solution was washed with H_2O and evaporated, giving needles m.p. 127–128° (hexane–acetone). ν_{max}^{KBr} (cm^{-1}): 3350, 1770, 1710, 1605, 1570, 1165.

5′,6′-*Dihydro*-8-*hydroxy*-6′,6′-*dimethylpyrano*-(2′,3′–7,6)-*coumarin* (IIIb). (1) *Preparation from brosiparin.* To a solution of brosiparin (360 mg) in AcOH (3·5 ml) 55% HI (2·3 ml) was added, and the mixture maintained under reflux (3 hr, N_2) in the dark. The cooled reaction mixture was poured onto ice. The precipitate was separated by filtration, washed with H_2O and dried. Its $CHCl_3$ soln, filtered through silica, gave needles (80 mg), m.p. 206–208° (hexane–$CHCl_3$). λ_{max}^{EtOH} (nm): 268, 330 (log ϵ 3·97, 4·21), $\lambda_{max}^{EtOH+NaOH}$ (nm): 283, 340 (log ϵ 4·22, 3·98). ν_{max}^{KBr} (cm^{-1}): 3400, 1710, 1618, 1567, 1149. NMR ($CDCl_3$, τ): 2·35 (*d*, *J* 10·3 Hz, H-4), 3·15 (*s*, H-5), 3·75 (*d*, *J* 10·3 Hz, H-3), 7·15 (*t*, *J* 6·8 Hz, Ar–C$\underline{H}_2$), 8·10 (*t*, *J* 6·8 Hz, C$\underline{H}_2$), 8·60 (*s*, two C$\underline{H}_3$). The *methyl ether* (IIIc) was obtained by reflux (8 hr) of IIIb in acetone with Me_2SO_4–K_2CO_3, as white needles, m.p. 99° (CCl_4). (2) *Synthesis* according to the procedure of Seshadri *et al.*[10] To a stirred suspension of dry daphnetin (3·56 g) in dioxane containing $BF_3.Et_2O$ (1 ml), 2-methyl-3-buten-2-ol (1·72 g) was added dropwise. After additional stirring at room temp. (1 hr), was the mixture filtered to remove unreacted daphnetin, diluted with moist Et_2O (50 ml), washed with H_2O (3 × 25 ml) and extracted with aq 1% Na_2CO_3 (3 × 25 ml). The alkaline soln was acidified and extracted with $CHCl_3$. The $CHCl_3$ soln. was evaporated and the residue chromatographed on silica, giving IIIb, m.p. and m.m.p. with a sample prepared from brosiparin 206–208° (hexane–$CHCl_3$).

Luvangetin (Ic). Needles, m.p. 108–109° (CCl_4) (lit.[12] m.p. 108–109°). NMR and MS data as required by lit.[17] *Dihydroluvangetin* (IIIc). Hydrogenation (10% Pd/C, AcOH–EtOH, 1:1) of Ic gave IIIc, needles, m.p. and m.m.p. with synthetic IIIc (see above) 99° (lit.[12] m.p. 131°, see Discussion). λ_{max}^{EtOH} (nm): 250, 260, 330 (log ϵ 3·69, 3·72, 4·20). ν_{max}^{KBr} (cm^{-1}): 1725, 1617, 1568, 1404, 1148, 820.

Brosiprenin (IVa). Yellow needles, m.p. 132–134° (hexane-acetone). M found: 328·1671; $C_{20}H_{24}O_4$ requires: 328·1674. λ_{max}^{EtOH} (nm): 261, 317 (log ϵ 4·11, 4·13); $\lambda_{max}^{EtOH+NaOH}$ (nm): 284, 332 (log ϵ

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4·29, 3·96). ν_{max}^{KBr} (cm^{-1}): 3500, 1733, 1600, 1577, 1451, 1152, 826. NMR ($CDCl_3$, τ): 2·08 (*d*, *J* 10·3 Hz, H-4), 3·60 (*d*, *J* 10·3 Hz, H-3), 4·90 (*m*, 2 =CH), 6·00 (*s*, OCH_3), 6·50 (*m*, 2 CH_2), 8·20 (*s*, two CH_3), 8·30 (*s*, two CH_3). MS: M 328 (40%), *m/e* (%) 297 (7), 285 (4), 273 (19), 272 (90), 258 (17), 257 (100), 243 (13), 230 (16), 225 (47), 297 (19). *Acetate* (IVb). Oil. ν_{max}^{film} (cm^{-1}): 1786, 1742, 1600, 1439, 1186. RMN (CCl_4, τ): 1·90 (*d*, *J* 10·3 Hz, H-4), 3·60 (*d*, *J* 10·3 Hz, H-3), 4·9 (*m*, two =CH), 6·05 (*s*, OCH_3), 6·5 (*m*, two CH_2), 7·54 (*s*, $OCOCH_3$), 8·20 (*s*, two CH_3), 8·30 (*s*, two CH_3).

Conversion of brosiparin (IIb) *into brosiprenin* (IVa). (1) *Prenylation of brosiparin.* To a solution of IIb (306 mg) in dry acetone (80 ml) were added anhydrous K_2CO_3 (225 mg) and 3,3-dimethylallyl bromide (200 mg). The mixture was heated under reflux (16 hr), cooled and filtered. The solvent was evaporated and the residue chromatographed on silica giving *O*-prenylbrosiparin (IId, 228 mg) as an oil. λ_{max}^{EtOH} (nm): 225, 302 (log ϵ 3·87, 4·02). ν_{max}^{film} (cm^{-1}): 1733, 1613, 1570, 1449, 1408, 1370. NMR (CCl_4, τ): 2·20 (*d*, *J* 10·3 Hz, H-4), 2·95 (*s*, H-5), 3·70 (*d*, *J* 10·3 Hz, H-3), 4·4 (*m*, =CH), 4·6 (*m*, =CH), 5·32 (*d*, *J* 7·7 Hz, OCH_2), 6·00 (*s*, OCH_3), 6·65 (*d*, *J* 7·7 Hz, CCH_2), 8·25 [*s*, two $C(CH_3)_2$]. (2) *Claisen rearrangement of* 8-*O*-*prenyl-brosiparin.* A solution of IId (132 mg) in *N,N*-dimethylaniline (4 ml) was heated under reflux (6 hr). The cooled reaction mixture was separated by TLC into brosiprenin (45 mg), m.p. and m.m.p. with natural IVa 132–134°, brosiparin (IIb, 45 mg) and *N,N*-dimethylaniline.

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