3β-HYDROXY-28-*P*-COUMAROYLOXY-LUP-20(29)-EN-27-OIC ACID FROM *CARAIPA DENSIFOLIA*

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ABSTRACT.—*Caraipa densifolia* Mart. (Clusiaceae) has afforded the new lupene derivative 3β-hydroxy-28-*p*-coumaroyloxy-lup-20(29)-en-27-oic acid (**8**), whose structure was deduced by chemical correlation with betulin (**6**). Simiarenol (**1**), taraxerone (**2**), friedelin (**3**), lupeol (**4**), betulinic acid (**5**), betulin (**6**), and β-sitosterol-β-D-glucoside (**7**) were also isolated in this study.

Extracts of the stem bark and twigs of *Caraipa densifolia* Mart. (Theaceae) displayed weak (ED_{50} 1.5 μg/ml) anticancer activity in the P-388 test system (1), but we were unable to continue to monitor this activity on extended fractionation. In the course of this work a number of common triterpenes were isolated including a new compound in the lupane series.

The genus *Caraipa* in the family Theaceae is comprised of approximately 20 species distributed principally in the tropical and sub-tropical New World (2). There is very little ethnomedical information available concerning *Caraipa* species, and our search produced only one brief reference to the oral use of the seeds of *C. lacerdaei* Rodr. and of *C. psidiformis* as an antihelmintic (3). No *in vitro*, *in vivo*, *in situ* or human experimental studies could be found, but several reports have appeared, principally from Gottlieb's laboratory, concerning the isolation of triterpenes and steroids (4–7) and xanthane derivatives (4–6,8) from plants in this genus.

EXPERIMENTAL[2]

PLANT MATERIAL.—The stem bark and twigs of *Caraipa densifolia* Mart. (Theaceae) used in this study were collected in Pennsylvania in July 1977. A voucher specimen representing the collection is deposited in the herbarium of the National Arboretum, U.S. Department of Agriculture, Washington D.C.

EXTRACTION AND FRACTIONATION.—The dried and milled stem bark and twigs (1.5 kg) were extracted successively with light petroleum and methanol. Evaporation of the methanol extract *in vacuo* afforded a residue which was partitioned between water and chloroform to afford, after processing, a chloroform soluble fraction (27.53 g).

CHROMATOGRAPHIC SEPARATION.—The chloroform soluble fraction (27.5 g) was chromatographed on a column of silica gel[3] (800 g, 70–230 mesh). Elution with chloroform, chloroform-methanol (97:3), chloroform-methanol (9:1), chloroform-methanol (3:1) and methanol gave fractions 1–5, 6–7, 8, 9 and 10, respectively.

ISOLATION AND IDENTIFICATION OF SIMIARENOL (**1**).—Fraction 1 (0.210 g), on crystallization from chloroform-light petroleum (1:1) at 0°, yielded simiarenol (**1**) as white crystals (0.025 g, 0.001%), mp 210°, $[\alpha]^{26}D+51°$ (c 0.4, $CHCl_3$) [Lit. (9) mp 210°, $[\alpha]D+50°$]. The isolate was identical with an authentic sample (mixed mp, ir and tlc).

ISOLATION AND IDENTIFICATION OF TARAXERONE (**2**).—The filtrate from fraction 1, on further standing at 0° for a week, yielded taraxerone (**2**) as white crystals (0.020 g, 0.001%), mp 239°, $[\alpha]^{26}D+14°$ (c 0.3, $CHCl_3$) [Lit. (10) mp 240°, $[\alpha]D+12°$]. The isolate was identical with an authentic sample (mixed mp, ir and tlc).

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[2]Melting points were determined on a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G spectrophotometer and the ir spectra with a Beckman model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}; absorption bands are recorded in wave numbers (cm^{-1}). Proton magnetic resonance spectra were recorded in $CDCl_3$ with a Varian T-60A instrument equipped with a Nicolet Model TT-7 Fourier Transform attachment. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ (ppm). Mass spectra were obtained with a Varian MAT-112S double focusing spectrometer operating at 70 eV.

[3]E. Merck, Darmstadt, West Germany.

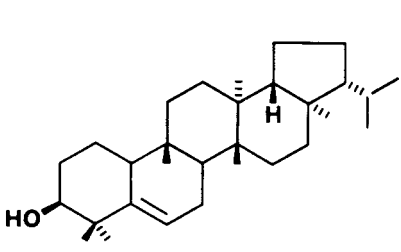
ISOLATION AND IDENTIFICATION OF FRIEDELIN (3).—The filtrate, after removal of **2**, was evaporated *in vacuo*. Crystallization of the residue from light petroleum yielded friedelin (**3**) as white needles (0.023 g, 0.001%), mp 264°, $[\alpha]^{26}D-19°$ (c 0.5, $CHCl_3$) [Lit. (11) mp 261–264°, $[\alpha]D-21°$]. The isolate was identical with an authentic sample (mixed mp, ir and tlc).

ISOLATION AND IDENTIFICATION OF LUPEOL (4).—Fraction 2 (1.85 g), on crystallization from hexane, afforded lupeol (**4**) as white crystals (0.110 g, 0.007%), mp 212°, $[\alpha]D+32°$ (c 0.5, $CHCl_3$) [Lit. (12) mp 215°, $[\alpha]D+33°$]. The isolate was shown to be identical with an authentic sample (mixed mp, ir and tlc).

ISOLATION AND IDENTIFICATION OF BETULINIC ACID (5).—Fraction 5 (3.580 g), on standing in chloroform (40 ml) at 0°, deposited a white solid, which, on crystallization from methanol, afforded betulinic acid (**5**) as white crystals (0.155 g, 0.01%), mp 317°, $[\alpha]D+12°$ (c 0.1, $CHCl_3$) [Lit. (13) mp 317°, $[\alpha]D+12°$]. The isolate was shown to be identical with an authentic sample (mixed mp, ir and tlc).

ISOLATION AND IDENTIFICATION OF BETULIN (6).—Fraction 7 (4.11 g), on standing in chloroform (25 ml) at 0°, afforded a white solid which, after preparative tlc on silica gel eluting with 2% methanol-chloroform and crystallization from hexane-methylene chloride (9:1), afforded betulin (**6**) as white crystals (0.048 g, 0.003%), mp 261°, $[\alpha]^{26}D+21°$ (c 0.2, $CHCl_3$) [Lit. (12) mp 261° $[\alpha]D+20°$]. The isolate was shown to be identical with an authentic sample (mixed mp, ir and tlc).

ISOLATION AND IDENTIFICATION OF β-SITOSTEROL-β-D-GLUCOSIDE (7).—Fraction 8 (2.3 g), when chromatographed on a column of silica gel (60 g, 70–230 mesh) eluted with chloroform, chloroform-methanol (97:3) and chloroform-methanol (19:1), afforded 3 fractions. The last fraction (1.6 g) on crystallization from chloroform yielded β-sitosterol-β-D-glucoside (**7**) as a white powder (0.045 g, 0.003%), mp 292° dec, $[\alpha]D-42°$ (c 0.4, pyridine) [Lit. (14) mp 290° dec, $[\alpha]D-41°$]. The isolate was shown to be identical with an authentic sample (mixed mp, ir and tlc).



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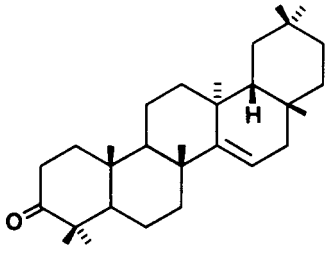

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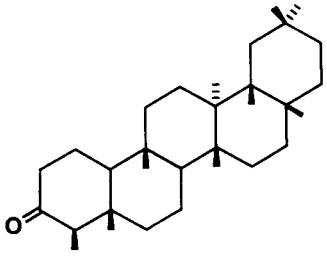

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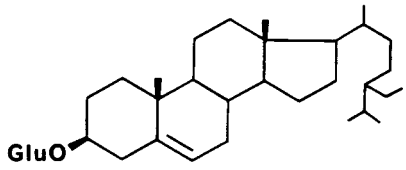

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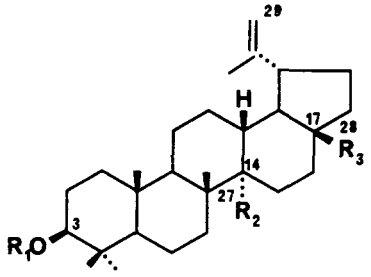
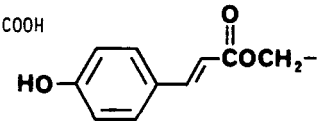
ISOLATION OF 3β-HYDROXY-28-*p*-COUMAROYLOXY-LUP-20(29)-EN-27-OIC ACID (8).—Large scale extraction of *C. densifolia* (36.3 kg) under the conditions described previously afforded a chloroform soluble fraction (369 g). Chromatography of this fraction (300 g) on a column of silica gel (3 kg, mesh 70–230) and elution with chloroform and chloroform-methanol mixtures of increasing polarity afforded 49 fractions. Fractions 39–45 (1.1 g) eluted with chloroform-methanol (19:1) were rechromatographed on Florisil[4] (30 g) eluted with chloroform, chloroform-methanol (49:1) and chloroform-methanol (19:1) to afford 3 fractions. The last fraction, on crystallization from diethyl ether-hexane (1:1), gave 3β-hydroxy-28-*p*-coumaroyloxy-lup-20(29)-en-27-oic acid (**8**) as a white powder (0.037 g, 0.0001%), mp 179–180°, $[\alpha]D-3.6°$ (c 0.8, acetone); uv, λ max (MeOH) 304 (log ε 4.31), 291 (sh) (4.21) and 227 nm (4.03), λ max (MeOH/KOH) 360, 302 (sh) and 240 nm; ir, ν max (KBr) 3350, 2930, 2875, 1710, 1695, 1632, 1510, 1450, 1375, 1250, 1162, 1020, 982, and 832 cm^{-1}; nmr, ($(CD_3)_2CO$), δ 7.61 (1H, d, $J=16$ Hz), 7.50 (2H, m, $J=8.6$

[4]Fisher Scientific Co., Chicago, Il. U.S.A.

Hz), 6.87 (2H, m, J=8.6 Hz), 6.33 (1H, d, J=16 Hz), 4.74 (1H, bd s, 29–H), 4.60 (3H, m, 29–H and 28–H_2), 3.17 (1H, dd, J=6.5, 8.5 Hz, 3–H), 1.72 (3H, bd s, 20–CH_3), 1.51 (m, –CH_2–), 1.02, 0.93, 0.89 and 0.76 (3H each, s, four tert. CH_3); ms, m/z 618 (M^+, $C_{39}H_{54}O_6$, 1%), 600 (1), 559 (2), 455 (5), 454 (19), 441 (4), 439 (3), 436 (6), 423 (2), 421 (2), 393 (3), 313 (1), 300 (2), 285 (2), 246 (6), 245 (3), 234 (10), 233 (3), 232 (3), 220 (3), 215 (3), 208 (4), 207 (20), 206 (4), 203 (3), 201 (5), 189 (18), 175 (7), 164 (14), 147 (94), 135 (18), 119 (16), 107 (18), 91 (19), 81 (25), 69 (24), 55 (25), 41 (22), 32 (30) and 28 (100).

HYDROLYSIS OF **8**.—Treatment of **8** (0.018 g) with 3% KOH in 95% ethyl alcohol-water (10 ml) under reflux for 4 hr afforded, after crystallization from chloroform-hexane (1:9), 3β,28-dihydroxy-lup-20(29)-en-27-oic acid (**9**, 0.013 g) as white crystals, mp 282–3°, $[\alpha]_D$+28.6° (c 0.38, $CHCl_3$); Rf 0.46 (diethyl ether); ir, ν max (KBr) 3480, 3320, 2955, 2878, 1698, 1646, 1452, 1387, 1367, 1180, 1022 and 885 cm^{-1}; nmr, (C_5D_5N) δ 4.92, 4.75 (1H each, bd s, 29–H_2), 4.60, 4.18 (2H, AB, J=12.0 Hz, 28–H_2), 3.38 (1H, dd, J=7.1, 8.2 Hz, 3–H), 1.77 (3H, bd s, 20–CH_3), 1.16, 1.13, 0.99 and 0.88 (3H each, s, four tert. CH_3); ms, m/z 472 (M^+, $C_{30}H_{48}O_4$, 4%), 454 (10), 441 (24), 423 (14), 246 (10), 234 (11), 207 (36), 189 (41), 187 (26), 175 (19), 173 (14), 160 (13), 147 (17), 135 (49), 123 (20), 121 (29), 119 (31), 105 (30), 95 (42), 81 (53), 69 (48), 41 (53), and 28 (100).



	R_1	R_2	R_3
4	H	CH_3	CH_3
5	H	CH_3	COOH
6	H	CH_3	CH_2OH
8	H	COOH	
9	H	COOH	CH_2OH
10	CH_3CO	COOH	CH_2OCOCH_3
11	H	COOH	CH_2OCOCH_3
12	H	CH_2OH	CH_2OH
15	CH_3CO	COOH	CH_2OH
16	H	CH_2OH	CH_3
17	CH_3CO	CH_2OCOCH_3	CH_3
18	CH_3CO	CH_2OH	CH_3

ACETYLATION OF 3β,28-DIHYDROXY-LUP-20(29)-EN-27-OIC ACID (**9**).—Compound **9** (0.0042 g) was treated with acetic anhydride and pyridine (1:10, 2 ml) at room temperature overnight. Work-up in the usual way afforded a mixture of two compounds, which, by preparative tlc eluting with chloroform-methanol (49:1), furnished two acetates as amorphous gums.

The less polar compound was identified as 3β,28-diacetoxy-lup-20(29)-en-27-oic acid (**10**, 0.0024 g); ir, ν max 3200, 2940, 1738, 1700, 1645, 1475, 1460, 1388, 1260, 1240, 1038, and 758 cm^{-1}; nmr, ($CDCl_3$), δ 4.72, 4.62 (1H each, bd s, 29–H_2), 4.43 (2H, m, 28–H_2), 2.07, 2.03 (3H each, s, 3, 28–$OCOCH_3$), 1.69 (3H, s, 20–CH_3), 1.39 (m, –CH_2–), and 0.97 (3H), 0.87 (3H), 0.83 (6H, four tert. CH_3); ms, m/z no M^+, 497 (M^+ –CH_3COO, 3%), 496 (9), 483 (2), 454 (2), 453 (2), 437 (16), 393 (4), 354 (1), 299 (2), 276 (4), 255 (4), 249 (6), 246 (6), 217 (5), 213 (5), 201 (13), 189 (67), 187 (25), 173 (15), 159 (10), 145 (14), 135 (28), 119 (24), 95 (30) and 43 (100).

The more polar compound was identified as 3β-hydroxy-28-acetoxy-lup-20(29)-en-27-oic acid (**11**, 0.0007 g), Rf 0.04 (5% MeOH–$CHCl_3$); ms, *m/z* 514 (M^+, $C_{32}H_{50}O_5$, 2%), 496 (3), 481 (3), 455 (3), 454 (9), 441 (3), 437 (5), 247 (5), 246 (13), 234 (7), 233 (8), 232 (8), 227 (6), 213 (8), 207 (60), 189 (56), 187 (39), 175 (22), 173 (20), 171 (12), 149 (12), 145 (27), 135 (43), 119 (49), 107 (54), 91 (52), 69 (88) and 41 (100).

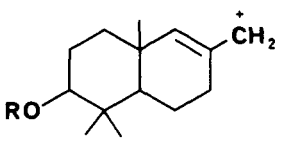
METHYLATION AND $LiAlH_4$ REDUCTION OF 3β,28-DIACETOXY-LUP-20(29)-EN-27-OIC ACID (**10**).—Compound **10** (0.002 g) was treated with excess ethereal diazomethane[5] at 0° for 3 days. Work-up in the usual way afforded a gum, which was treated with excess $LiAlH_4$ in dry ether under reflux for 24 hr. Standard work-up afforded 3β,27,28-trihydroxy-lup-20(29)-ene (**12**), Rf 0.54 (8% methanol-chloroform); ir, ν max (NaCl plate) 3350, 2940, 2870, 1650, 1457, 1385, 1010 and 745 cm^{-1}; ms, *m/z* 458 (M^+, $C_{30}H_{50}O_3$, 2%), 440 (3), 428 (12), 427 (35), 409 (22), 391 (4), 287 (2), 285 (2), 273 (5), 269 (3), 259 (5), 255 (6), 247 (6), 234 (6), 219 (7), 207 (30), 201 (18), 189 (30), 187 (21), 175 (24), 173 (15), 163 (16), 147 (20), 135 (44), 95 (66), 55 (98) and 43 (100).

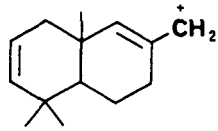
CONVERSION OF 3β,27,28-TRIHYDROXYLUP-20(29)-ENE (**12**) TO BETULIN (**6**).—Compound **12** (0.0015 g) was treated with acetic anhydride and pyridine (1:10, 2 ml) at room temperature overnight. Work-up in the usual way afforded a gum (0.002 g) which was refluxed with *p*-toluene sulfonyl chloride (0.004 g) in pyridine (1.5 ml) for 24 hr. The amorphous product (0.0025 g) was then refluxed with $LiAlH_4$ (0.007 g) in dry freshly distilled THF (3 ml) for five days. The product was separated preparatively on silica gel eluted with chloroform-methanol (99:1) to afford betulin (**6**) as a white powder, mp 257° [Lit. (12) mp 261°]; ir, ν max (KBr) 3400, 2972, 2937, 1645, 1460, 1375, 1022 and 880 cm^{-1}; ms, *m/z* 442 (M^+, 18%), 411 (31), 234 (23), 233 (11), 220 (15), 208 (11), 207 (48), 205 (13), 204 (13), 203 (39), 191 (24), 189 (61), 177 (20), 175 (24), 161 (20), 149 (16), 147 (30), 145 (20), 135 (50), 121 (53), 109 (50), 107 (60), 105 (38), 95 (97) and 55 (100). The reaction product was identical with an authentic sample of betulin (**6**) (mixed mp, ir, mass and tlc).

STRUCTURE ELUCIDATION OF 3β-HYDROXY-28-*p*-COUMAROYLOXY-LUP-20(29)-EN-27-OIC ACID (**8**).—A positive Liebermann-Burchard test (15) was obtained for the isolate which showed a molecular ion at *m/z* 618 in agreement with a molecular formula of $C_{39}H_{54}O_6$. The uv spectrum and large (56 nm) bathochromic shift on the addition of base indicated a *p*-hydroxycinnamoyloxy group to be present in the compound. α,β-Unsaturated ester (1710 cm^{-1}), tertiary COOH (1695 cm^{-1}), hydroxy (3350 cm^{-1}) and terminal double bond (1646 and 885 cm^{-1}) functionalities were deduced from the ir spectrum.

Base hydrolysis of **8** afforded a dihydroxy acid displaying a molecular ion at *m/z* 472 ($C_{30}H_{48}O_4$), indicating a loss of $C_9H_7O_3$ equivalent to the hydrolysis of a hydroxycinnamic acid group. In agreement with this, the two *trans* olefinic protons and the two sets of *ortho*-coupled aromatic protons observed in the nmr spectrum of **8** were now absent in the nmr spectrum of the hydrolysis product.

The nmr spectrum of **8** also revealed an axial proton (δ 3.17), as a doublet of doublets (J=8.5, 6.5 Hz), of a secondary hydroxy group and a three-proton broad multiplet (δ 4.60) for the methylene group of an esterified primary alcohol, and one of the geminal olefinic protons. A second such proton was found at δ 4.74. For the hydrolysis product **9**, an AB system of the primary alcohol was now observed at δ 4.18. This upfield (Δδ –0.42) shift indicated the esterifying group to be attached to the primary and not the secondary alcoholic group.





13 R = H, m/z 207

15 R = $COCH_3$ m/z 249

14 m/z 189

Methylation of **9** with diazomethane and subsequent reduction with $LiAlH_4$ afforded a triol of molecular weight 454 ($C_{30}H_{50}O_3$) confirming the presence of a carboxylic acid group in **9**. The location of this group was deduced from the mass spectral fragmentation of **8**, **9**, **11** and **12** which gave the characteristic (16) fragment **13** at *m/z* 207 and fragment **14** at *m/z* 189. Similarly **10** gave the fragment **15** at *m/z* 249 and **14** at *m/z* 189. The primary alcohol and –COOH groups should, therefore, be attached at C–27 and C–28 and not to the A or B rings.

Acetylation of **9** afforded a mixture of two acetates (**10** and **11**) in the ratio 3:1. The high yield of the diacetate **10**, and the absence of any C–3 only acetylated product, such as **15**, is to be contrasted with yields from the acetylation of 3β,27-dihydroxy-lup-20(29)-ene (16) which afforded **17** and **18** in the ratio 1:3 (17). This indicates that in the isolate from *C. densifolia* the primary alcohol (hydroxy methyl) group is attached not at the hindered C–14 position, but rather at the less hindered, equatorial C–28 position, leaving the carboxylic acid residue attached at C–17.

[5]Prepared from Diazald®, Aldrich Chemical Co., Milwaukee, WI, U.S.A.

Chemical correlation established the carbon framework of **8**. Acetylation of **12** followed by vigorous tosylation and $LiAlH_4$ reduction of the product yielded betulin (**6**). The formation of **6**, considered in conjunction with the data discussed above, indicated the isolate **8** to possess the structure 3β-hydroxy-28-*p*-coumaroyloxy-lup-20(29)-en-27-oic acid.

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