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BETULIN-3-CAFFEATE FROM *QUERCUS SUBER*. ^{13}C-NMR SPECTRA OF SOME LUPENES

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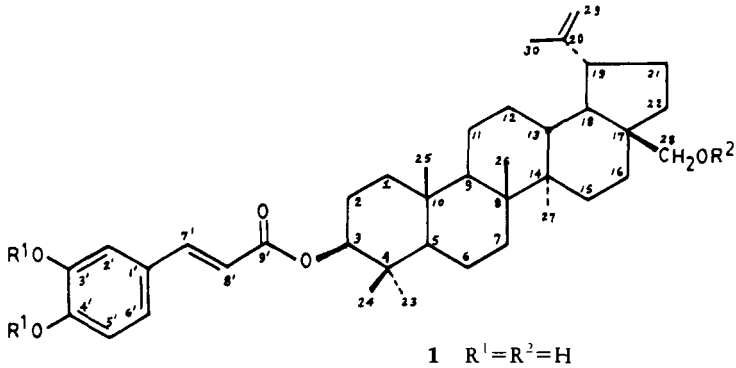
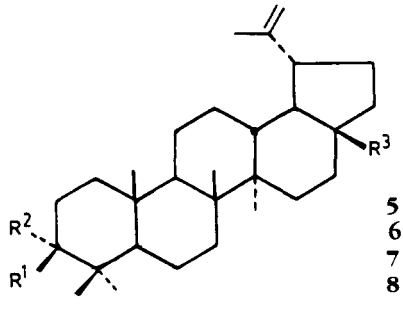
ABSTRACT.—A new α-lupene ester, lup-20(29)-en-28-ol-3β-yl caffeate (betulin-3-caffeate) [**1**], has been isolated from cork waste along with betulin, betulonic acid, and betulinic acid. ^{13}C-nmr signal assignments of **1** and its derivatives are reported.

Cork waste (*Quercus suber* L., Fagaceae) was found to be a rich source of triterpenoids (1). Our investigation has led to the isolation of four α-lupene derivatives, one of which is the new α-lupene ester, lup-20(29)-en-28-ol-3β-yl caffeate [**1**]. Betulin [**5**], betulonic acid [**7**], and betulinic acid [**8**] are the known components isolated (2). Their structures have been established from various spectral (uv, ir, ^{1}H and ^{13}C nmr, mass) and chemical characteristics.

The new, somewhat air-sensitive, α-lupene ester gave positive Liebermann-Burchard and tetranitromethane color reactions for triterpenoids and olefinic unsaturation, respectively, and showed uv absorptions undergoing strong bathochromic shifts upon addition of aqueous alkali. Absorptions for hydroxyls, conjugated ester, and terminal double bond in the ir spectrum were also noted. Compound **1** readily formed a triacetate **2**, $C_{45}H_{62}O_8$ ($[M]^+$ 730). Two of the acetylable hydroxyl groups underwent methylation, inasmuch as the dimethyl ether **3** formed with CH_2N_2 yielded a monoacetate **4**. The presence of a lupene framework having an angular hydroxymethyl group and a caffeate moiety in **1** was suggested by the inspection of the ^{1}H- and ^{13}C-nmr spectra of **1**–**4** and mass spectrum of **2** as well as by chemical correlation of both **1** and **2** with betulin [**5**].



1 $R^1=R^2=H$
2 $R^1=R^2=Ac$
3 $R^1=Me$, $R^2=H$
4 $R^1=Me$, $R^2=Ac$



5 $R^1=OH$, $R^2=H$, $R^3=CH_2OH$
6 $R^1=OAC$, $R^2=H$, $R^3=CH_2OAc$
7 $R^1,R^2=O$, $R^3=COOH$
8 $R^1=OH$, $R^2=H$, $R^3=COOH$

The 300 MHz ^{1}H-nmr spectrum of **1** displayed signals for an isopropylene function (δ 4.68 and 4.58, 1H each, br s and 1.68, 3H, s), five *tert*-CH_3 groups [δ 1.01 (3H, s, H_3-26), 0.98 (3H, s, H_3-27), 0.90 (3H, s, H_3-24), 0.88 (3H, s, H_3-23), and 0.87 (3H, s, H_3-25)] and a -CH_2C*H*-OCOR function (δ 4.58, 1H, m, *H*-3), besides signals for a set of geminal protons at δ 3.82 and 3.35 (1H each, d, J_{gem}= 10.8 Hz, H_2-28) and an allylic proton at δ 2.38 (1H, m, *H*-19) for the aforesaid triterpenoid skeleton. Similar signals for magnetically nonequivalent geminal protons were also present in the spectrum of **3**, and they underwent downfield shift (Δδ +0.45–0.50 ppm) upon acetylation of **1** and **3** to **2** and **4**, respectively, in conformity with their association with a hydroxymethyl group. Again, ^{1}H resonances for **1** at δ 6.25 (1H, d, J= 15.8 Hz, *H*-8′), 6.86 (1H, d, $J \approx$ 7.8 Hz, *H*-5′), 6.99 (1H, br d, $J \approx$ 7.8 Hz, *H*-6′), 7.09 (1H, br s, *H*-2′), and 7.54 (1H, d, J= 15.8 Hz, *H*-7′) were in conformity with the presence of a 3′,4′-dihydroxycinnamyloxy moiety. Alkaline (2% methanolic KOH) hydrolysis of both **1** and **2** gave betulin [**5**], identical with a natural specimen, which further established the oxygenation pattern on the α-lupene skeleton.

The location of the caffeyl moiety at C-3 in the new compound was established from mutual comparison of ^{1}H- and ^{13}C-nmr spectra of **1**–**4**, along with those of betulin [**5**] and betulin diacetate [**6**] as appropriate. Similar resonance positions for the -CH_2OH group in **1**, **3**, and **5** (δ 3.81 and 3.34, 1H each, d, J_{gem}= 11.0 Hz) on one hand and for the -CH_2OAc group in **2**, **4**, and **6** (δ 4.27 and 3.84, 1H each, d, J_{gem}= 10.9 Hz) on the other were noted. Again, ion peaks (3) of **2** at *m/z* (%) 466 $[M-(AcO)_2C_6H_3$-$CH=CH-COOH]^+$ (27), 247 (10) 205 $[247 - CH_2CO]^+$ (58), 187 $[247 - HOAc]^+$ (37), 175 $[247 - CH_2OAc+H]^+$ (22), and 423 $[466 - CH_3CO]^+$ (10) strongly supported the location of an acetoxy function at C-28 on the α-lupene framework. Appearance of the H-3 resonance (δ 4.58, m) in **2** at a slightly different field from that in **6** (δ 4.48, m) was in conformity with the attachment of the caffeate moiety at C-3 as in **2**, and its equatorial (β) orientation was evident from the nature and width of the H-3 multiplet as well as from a comparison of carbon resonances of ring A as well as *gem*-dimethyl carbons in **1**–**4** with **5** and **6**. The carbon shift assignments for **1**–**4**, **6**, **7** given in Table 1 followed from consideration of the spectrum of betulin [**5**] (4). Heteronuclear chemical shift correlation studies established the carbon resonance assignments, in particular of the caffeate moiety in **2**, and proton resonances associated with various methyl groups. Incidentally, this is only the second report of the occurrence of a caffeyl ester of lupene from a natural source (5).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were uncorrected. Ir spectra and rotations were taken in $CHCl_3$; ^{1}H-nmr (300 MHz) and ^{13}C-nmr (75 MHz) spectra in $CDCl_3$. Proton noise decoupling and attached proton test (APT) or DEPT experiments on Bruker AM 300L Supercon NMR spectrometer established carbon shifts and degree of protonation. The solvent served as internal lock and internal standard ($\delta_{TMS}=\delta_{CDCl_3}$+76.9 ppm). Chromatography was carried out using Si gel (BDH).

ISOLATION.—Air-dried crushed cork waste (3 kg) collected from a local dealer was exhaustively extracted by Soxhlet extractor with petroleum ether and $CHCl_3$ in succession for 84 h each. The petroleum ether insoluble residue (27 g) was leached with boiling Me_2CO, and the extracted yellow gummy residue (4 g) was chromatographed with solvents and solvent mixtures of increasing polarity. $CHCl_3$/MeOH fractions provided betulin (33 mg), betulonic acid (48 mg), and betulinic acid (160 mg) in succession. The Me_2CO-soluble fraction of the $CHCl_3$ extract of the plant was similarly chromatographed, and $CHCl_3$-MeOH (98:2) eluates gave a somewhat unstable gummy solid (8 g) that afforded some betulin-3-caffeate [**1**] after repeated tlc purification. The crude solid (4 g) was treated with Ac_2O (10 ml) and C_5H_5N (10 ml), warmed on a steam bath for 15 min, and kept at room temperature for 20 h. Work-up and rechromatography furnished acetyl α-lupene ester **2** (1 g).

LUP-20(29)-EN-28-OL-3β-YL CAFFEATE [**1**].—Amorphous, $[\alpha]^{25}D$ +33.6°; R_f 0.6 ($CHCl_3$-MeOH

TABLE. 1. Carbon-13 Chemical Shifts of Triterpenoids **1**–**7**.

Carbon	Compound						
	1[a]	**2**[b]	**3**[c]	**4**[d]	**5**(4)	**6**	**7**
C-1	38.3	38.3	38.4	38.3	38.7	38.3	39.5
C-2	23.7	23.6	23.8	23.6	27.3	23.6	34.0
C-3	81.0	81.0	80.8	80.9	78.9	80.8	218.0
C-4	38.0	37.9	38.0	37.8	38.8	37.7	47.2
C-5	55.4	55.3	55.4	55.3	55.2	55.3	54.9
C-6	18.1	18.0	18.1	18.0	18.2	18.1	19.5
C-7	34.1	34.4	34.1	34.3	34.2	34.4	33.5
C-8	40.9	40.8	40.9	40.8	40.9	40.8	40.6
C-9	50.3	50.2	50.3	50.2	50.4	50.2	49.8
C-10	37.0	36.9	37.1	36.9	37.1	37.0	36.8
C-11	20.8	20.7	20.8	20.8	20.8	20.8	21.3
C-12	25.1	25.0	25.1	25.0	25.2	25.1	25.4
C-13	37.3	37.4	37.3	37.4	37.3	37.5	38.4
C-14	42.7	42.5	42.7	42.6	42.7	42.6	42.4
C-15	27.0	26.9	27.0	26.9	27.0	27.0	30.5
C-16	29.5	29.5	29.1	29.2	29.1	29.6	32.0
C-17	46.2	46.2	47.7	46.2	47.7	46.2	56.3
C-18	48.7	48.6	48.7	48.6	48.7	48.7	49.1
C-19	47.7	47.5	47.7	47.5	47.7	47.6	46.8
C-20	150.3	149.9	150.3	150.1	150.3	150.0	150.2
C-21	29.7	29.5	29.7	29.6	29.7	29.6	29.6
C-22	33.9	34.0	33.9	34.0	33.9	34.1	36.8
C-23	27.9	27.8	27.9	27.8	27.9	27.8	26.5
C-24	16.5	16.5	16.7	16.5	15.3	16.4	20.9
C-25	16.1	16.0[e]	16.1	16.0[e]	16.0	16.0	15.8[e]
C-26	15.9	15.9[e]	15.9	15.9[e]	16.0	16.0	15.7[e]
C-27	14.7	14.6	14.7	14.6	14.7	14.6	14.5
C-28	60.5	62.6	60.5	62.6	60.5	62.7	181.7
C-29	109.6	109.7	109.6	109.7	109.6	109.8	109.6
C-30	19.0	18.9	19.0	18.9	19.0	19.0	19.2
$OCOCH_3$		171.3		171.0		170.9	
						170.9	
$OCOCH_3$		20.8		20.9		20.9	
						21.0	

[a]Other signals present are at δ 114.1 (C-5′), 115.2 (C-2′), 116.2 (C-8′), 122.2 (C-6′), 127.5 (C-1′) 143.8 (C-3′), 144.4 (C-7′), 146.2 (C-4′), and 167.5 (C-9′).

[b]Other signals present are at δ 20.4 (2 × Ar-$OCOCH_3$), 119.9 (C-8′), 122.5 (C-2′), 123.7 (C-5′), 126.1 (C-6′), 133.3 (C-1′), 142.1 (C-7′), 142.2 (C-3′), 143.2 (C-4′), 166.2 (C-9′), 167.7 and 167.8 (2 × Ar-$OCOCH_3$).

[c]Other signals present are at δ 55.8 (OCH_3), 55.9 (OCH_3), 109.7 (C-5′), 111.1 (C-2′), 116.5 (C-8′), 122.4 (C-6′), 127.5 (C-1′), 144.1 (C-7′), 149.1 (C-3′), 150.9 (C-4′), and 166.9 (C-9′).

[d]Other signals present are at δ 55.8 (2 × OCH_3), 109.7 (C-5′), 111.1 (C-2′), 116.5 (C-8′), 122.4 (C-6′), 127.5 (C-1′), 144.1 (C-7′), 149.4 (C-3′), 150.8 (C-4′), and 166.9 (C-9′).

[e]Values bearing same superscript are interchangeable.

85:15); λ max (EtOH) 221, 235, 244, 333 nm; λ max (EtOH+NaOH) 217, 380 nm; ν max 3530, 1690, 1630, 1600, 1520, 1475, 925 cm^{-1}.

3β-(3′,4′-DIACETOXYCINNAMYLOXY)-LUP-20(29)-EN-28-YL ACETATE [**2**].—$C_{45}H_{62}O_8$, mp 144–145° ($CHCl_3$/MeOH), $[\alpha]^{25}D$ +17.6°; R_f 0.5 ($CHCl_3$-MeOH 98:2); λ max (EtOH) 219, 281 nm (log ε 4.32, 4.42); λ max (EtOH+NaOH) 219, 266, 382 nm (log ε 4.15, 4.05, 4.43); ν max (KBr) 1775, 1735, 1708, 1638, 1500, 1450, 1420, 1380, 1360, 1315, 1200, 1170, 1010, 980, 885 cm^{-1}; ^1H nmr δ 7.59 (1H, d, J=15.9 Hz, *H*-7′), 7.39 (1H, dd, J=7.8, 2.0 Hz, *H*-6′), 7.36 (1H, br s, *H*-2′), 7.18 (1H, d, J=7.8 Hz, *H*-5′), 6.36 (1H, d, J=15.9 Hz, *H*-8′), 4.68 and 4.58 (1H each, br s, H_2-29), 4.58 (1H,

m, *H*-3), 4.27 and 3.85 (1H each, d, J_{gem}=11.0 Hz, H_2-28), 2.39 (1H, m, *H*-19), 2.29 (3H, s, Ar-OCOCH_3), 2.28 (3H, s, Ar-OCOCH_3), 2.06 (3H, s, OCOCH_3), 1.68 (3H, s, H_3-30), 1.04 (3H, s, H_3-26), 0.98 (3H, s, H_3-27), 0.90 (3H, s, H_3-24), 0.87 (6H, s, H_3-23 and H_3-25); ms *m/z* (%) $[M]^+$ 730 (1), 467 (11), 466 (27), 423 (10), 393 (7), 247 (10), 205 (58), 203 (26), 202 (13), 191 (27), 190 (33), 189 (79), 187 (37), 180 (46), 177 (11), 175 (22), 164 (11), 163 (91), 161 (22), 147 (25), 136 (41), 135 (46), 134 (36), 133 (30) (Found: C 73.76, H 8.41; $C_{45}H_{62}O_8$ requires C 73.93, H 8.55%). Hydrolysis of **2** (50 mg) with 2% methanolic KOH yielded betulin [**5**] (18 mg).

3β-(3′,4′-DIMETHOXYCINNAMYLOXY)-LUP-20(29)-EN-28-OL [**3**].—Amorphous, $[\alpha]^{25}D$ +19.0°; R_f 0.4 ($CHCl_3$); λ max (EtOH) 211, 233, 296, 323 nm; ^{1}H nmr δ 7.61 (1H, d, *J*=15.8 Hz, *H*-7′), 7.10 (1H, br d, *J*≈8.3 Hz, *H*-6′), 7.06 (1H, br s, *H*-2′), 6.87 (1H, d, *J*=8.3 Hz, *H*-5′), 6.31 (1H, d, *J*=15.8 Hz, *H*-8′), 4.69 and 4.59 (1H each, br s, H_2-29), 4.61 (1H, m, *H*-3), 3.92 (6H, s, 2×OCH_3), 3.81 and 3.34 (1H each, d, J_{gem}=11.1 Hz, H_2-28), 2.38 (1H, m, *H*-19), 1.69 (3H, s, H_3-30), 1.04 (3H, s, H_3-26), 0.99 (3H, s, H_3-27), 0.93 (3H, s, H_3-24), 0.90 and 0.88 (3H each, s, H_3-23 and H_3-25).

3β-(3′,4′-DIMETHOXYCINNAMYLOXY)-LUP-20(29)-EN-28-YL ACETATE [**4**].—Amorphous; ^{1}H nmr δ 7.60 (1H, d, *J*=15.9 Hz, *H*-7′), 7.10 (1H, br d, *J*≈8.3 Hz, *H*-6′), 7.06 (1H, br s, *H*-2′), 6.86 (1H, d, *J*=8.3 Hz, *H*-5′), 6.31 (1H, d, *J*=15.9 Hz, *H*-8′), 4.69, 4.59 (1H each, br s, H_2-29), 4.60 (1H, m, *H*-3), 4.25 and 3.85 (1H each, d, *J*=10.9 Hz, H_2-28), 3.91 and 3.90 (6H, s, 2×OCH_3), 2.44 (1H, m, *H*-19), 2.07 (3H, s, OCOCH_3), 1.68 (3H, s, H_3-30), 1.04 (3H, s, H_3-26), 0.98 (3H, s, H_3-27), 0.92 (3H, s, H_3-24), 0.89 and 0.87 (H_3-23 and H_3-25).

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