A New Triterpenic Acid from Schefflera impressa

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A NEW TRITERPENIC ACID FROM *SCHEFFLERA IMPRESSA*

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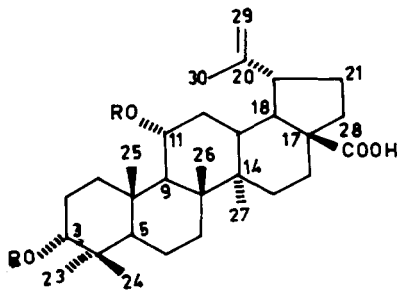
ABSTRACT.—A new triterpene, 3α,11α-dihydroxylup-20(29)-en-28-oic acid [**1**], has been isolated from the bark and stem of *Schefflera impressa*. Its structure has been deduced from spectroscopic data and chemical investigations.

In previous reports (1–3) we described the isolation and identification of twelve compounds including three new saponins from the stem and bark of *Schefflera impressa* C.B. Clarke (Araliaceae). The present communication describes the isolation and structure elucidation of a new triterpenic acid provisionally named impressic acid, 3α,11α-dihydroxylup-20(29)-en-28-oic acid [**1**], and three known compounds, stigmasterol, campesterol, and chrysophanol. Triterpene **1** is chemotaxonomically important because of its structural similarity with the main active constituent of *Schefflera octophylla* (4), which is used in Vietnamese folk medicine as a tonic, as an antirheumatic agent, and for liver diseases. Pharmacological investigations of impressic acid [**1**] are in progress.

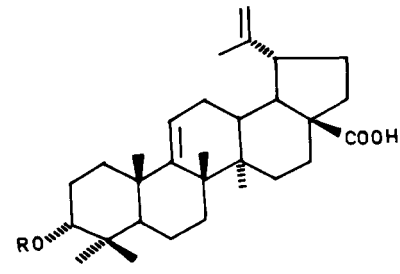
The stem and bark of *S. impressa* were extracted with MeOH, and the crude saponin fraction on chromatographic separation over Si gel afforded triterpene **1** ($C_{30}H_{48}O_4$) $[M]^+$ at *m/z* 472, mp 234–236°. Its ir spectrum (KBr) showed absorptions at 3474, 1705, 1639, and 886 cm^{-1} assignable to hydroxyl, carboxyl, and $>C=CH_2$ functions, respectively.

Acetylation of **1** gave diacetate **2** (mp 120–124°). This indicated that impressic acid contains two hydroxyl groups. The ^{1}H-nmr spectrum of **1** showed two olefinic protons at δ 4.59 and 4.72, five tertiary methyl groups, one vinylic at δ 1.67, and two secondary hydroxyl protons. The triplet-like signal centered at δ 3.32 ($W_{1/2}$ = 7 Hz) indicated that one hydroxyl group was present at the C-3 position which, on acetylation with Ac_2O and pyridine, shifted from δ 3.32 to 4.50 ($W_{1/2}$ = 7 Hz). Stereochemistry of the C-3 position is clear from the ^{1}H-nmr data with a $W_{1/2}$ of 7 Hz for the hydrogen geminal to the hydroxyl group, and an equatorial β-hydrogen and an axial α-hydroxyl group are required (5). Further evidence for this assignment was obtained from the ^{13}C-nmr spectrum. Kang (6) has shown that resonances of C-1, C-5, and C-24 are strongly influenced by the stereochemistry of the hydroxyl group at C-3.

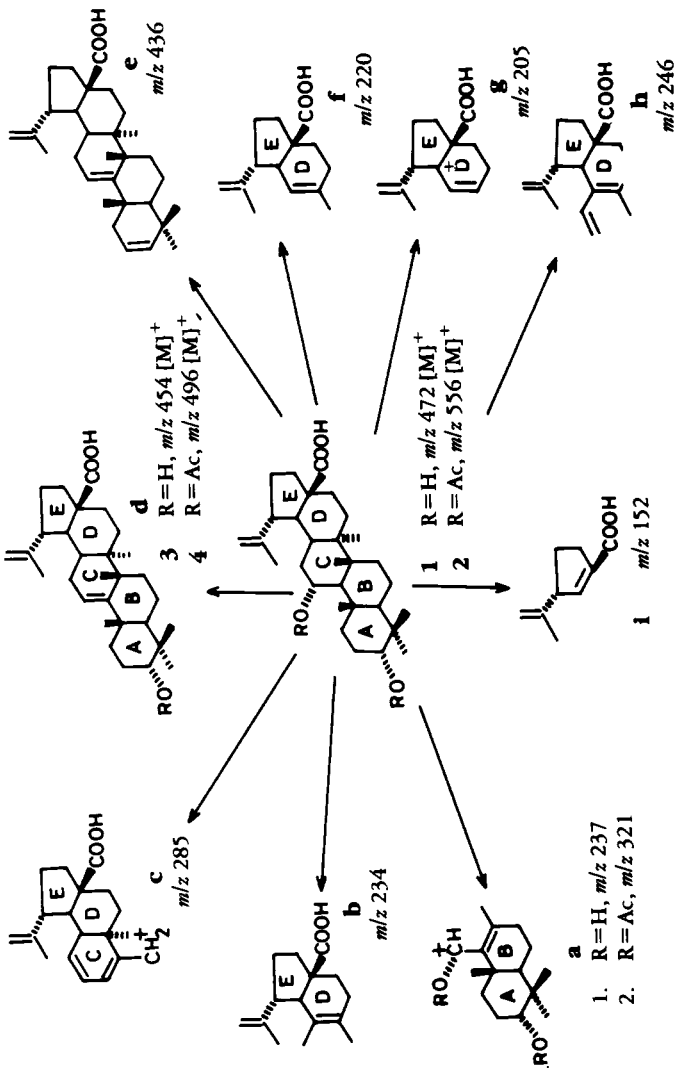
Conversion of the C-3 hydroxyl group from equatorial to axial stereochemistry re-



1 R=H
2 R=Ac



3 R=H
4 R=Ac



SCHEME 1. Mass fragmentation of 3α, 11α-dihydroxylup-20(29)-en-28-oic acid and its derivatives **1–4**.

sults in an upfield shift of C-1 and C-5 of ca. 6 ppm and C-24 shifts downfield by ca. 6 ppm, leaving C-23 almost unaffected (6).

On comparing the ^{13}C-nmr signals of **1** with those expected for C-3 equatorial and axial hydroxyl epimers, it was evident that impressic acid has an axial (α-oriented) hydroxyl group at C-3.

The observation that impressic acid [**1**] cannot be oxidized with periodic acid indicates that the second hydroxyl group is not at C-2. No acetonide was formed, but impressic acid was partly dehydrated to dehydroimpressic acid [**3**] mp 224–226° on stirring in Me_2CO containing a few drops of concentrated H_2SO_4. The ms of this compound ($[M]^+$ at *m/z* 454) indicated that only one hydroxyl group was eliminated as H_2O. The nmr spectrum showed only one additional olefinic proton signal, as a triplet at δ 5.22. The signal due to H-3β was present as a broad singlet at δ 3.35, which on acetylation with Ac_2O and pyridine (to 3α-acetoxydehydroimpressic acid [**4**] mp 190–192), shifted from δ 3.35 to 4.55. Thus, the other hydroxyl group, which is eliminated during acid-catalyzed dehydration, must be present on a ring other than A. Rahman *et al.* (7) reported that triterpenoids having unsubstituted D and E rings show characteristic fragment ions **f** − H, **g**, and **i** in their ms. The ms of **1** also showed similar fragments at *m/z* 152 [**i**], 205 [**g**], and 219 [**f** − H], indicating that rings D and E are not substituted. Thus, the position of the second hydroxyl group is restricted to ring B or ring C. It may be noted that the presence of a second hydroxyl group at C-6 in ring B would be expected to give rise to a peak at *m/z* 223 as observed in loranthol (8). This was not observed in the ms of impressic acid, but the appearance of an intense **a** type ion at *m/z* 237 indicated that a second hydroxyl group is present in ring C at C-11 (Scheme 1). This conclusion was supported by the fact that compounds containing the lup-20(29)-ene skeleton show a peak for C-11 at ca. 20.9 ppm in their ^{13}C-nmr spectra, but shifting of this signal to 69.8 ppm clearly indicated the presence of a hydroxyl group at C-11. The signal due to the C-11 proton in **1** appeared as a ddd centered at δ 3.90, which on acetylation to **2** shifted to a ddd centered at δ 5.19. This indicated that the 11-OH is in the α position. This was further supported by correlation of ^{13}C-nmr values of **1** with the substituent effect of a hydroxyl group at the 11α position of a steroidal skeleton (9). Besides the expected large downfield shift for the signals corresponding to C-11, signals at C-9, C-12, and C-1 were also significantly displaced downfield due to the 11α configuration of this hydroxyl group (10). The appearance of a signal at 179.2 ppm showed the presence of a free carboxylic group. Furthermore, ions at *m/z* 152 [**i**] 205 [**g**] and 219 [**f** − H] locate the carboxylic group at C-17 (*i.e.*, C-28) in **1** (7).

Stigmasterol, campesterol, and chrysophanol were identified on the basis of their mp, ir, ^{1}H nmr, ms, and co-tlc with authentic samples.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Toshniwal melting point apparatus and are uncorrected. Ir spectra were taken with a Perkin-Elmer 399B spectrometer. ^{1}H- (80 MHz) and ^{13}C- (20 MHz) nmr spectra were measured with a Varian FT 80A spectrometer. TMS was used as the internal standard, and chemical shifts were given in δ (ppm) values. Ms was determined on a JMXDX-300 spectrometer. Optical rotation was measured with a JASCO model DIP-181. Si gel 60–120 mesh (BDH) was used for cc and Si gel G or H (Merck) was used for tlc, preparative tlc, and vlc. Tlc spots were visualized by spraying with 10% H_2SO_4 followed by heating at 100°.

PLANT MATERIAL.—Plant material was collected from Darjeeling, India, and identified as *S. impressa* by Dr. S.P. Jain of the Botany Division. A voucher specimen (No. 1909) was deposited in the herbarium of this institute.

ISOLATION PROCEDURES.—Powdered air-dried stems and bark of *S. impressa* (2.3 kg) were extracted with MeOH at room temperature for 10 days. The extract was concentrated in vacuo, and the residue was dissolved in H_2O. The H_2O solution was extracted with *n*-hexane, C_6H_6, $CHCl_3$, and EtOAc, and finally

with *n*-BuOH saturated with H_2O. The *n*-BuOH fraction when concentrated under vacuum yielded a brown viscous mass (62.8 g), which was subjected to Si gel (725 g) cc and eluted with EtOAc with increasing amounts of MeOH. Fractions (250 ml each) were collected and monitored by tlc. The EtOAc eluate afforded a solid (0.28 g) which on further cc afforded **1** (0.20 g). The other eluates yielded saponins as reported earlier (1–3).

IMPRESSIC ACID [**1**].—White powder (0.20 g): mp 234–236° (MeOH); $[\alpha]^{25}D$ +2.6° (MeOH, c = 0.06); ir ν max (KBr) cm^{-1} 3473, 1705, 1639, 886 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.84, 0.90, 0.90, 0.98, 0.98 (s, Me-23, -24, -25, -26, -27), 1.67 (s, Me-30), 3.32 (br s, $W_{1/2}$ = 7 Hz, H-3β), 3.90 (six line pattern, J = 11 Hz, J' = 5 Hz, H-11β), 4.59 and 4.72 (2xm, H_2-29); eims *m/z* (rel. int.) $[M]^+$ 472 (23), $[M-H_2O]^+$ [**d**] 454 (57), $[M-2H_2O]^+$ [**e**] 436 (54), $[M-2H_2O-CO_2]^+$ 392 (25), [**c**] 285 (30), [**h**] 246 (29), [**a**] 237 (28), [**b**] 234 (98), 221 (24), [**f**] 220 (49), [**f**−H] 219 (75), [**g**] 205 (32), 203 (52), 201 (93), 189 (96), 175 (100), 161 (44), [**i**] 152 (31), 147 (59), 133 (80), 121 (86), 107 (99). Found C 70.91, H 9.95; calcd for $C_{30}H_{48}O_4 2H_2O$, C 70.86, H 10.23%. ^{13}C nmr see Table 1.

3α,11α-DIACETYLIMPRESSIC ACID [**2**].—A solution of **1** (50 mg) and Ac_2O/pyridine was allowed to stand at room temperature for 1 day. Cc on Si gel gave **2** (35 mg): mp 120–124°; ir ν max (KBr) 1735 (ester), 1700 (COOH), 1645 (>C=CH_2), 1248 (OAc) cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.78, 0.80, 0.90, 1.00, 1.20, (s, Me-23, -24, -25, -26, -27), 1.60 (s, Me-30), 1.92, 2.02, (2 × s, -OAc), 4.50 (br s, $W_{1/2}$ = 7 Hz, H-3β), 4.50 and 4.65 (2xm, H_2-29), 5.19 (six line pattern, J = 11 Hz, J' = 5 Hz, H-11β); eims *m/z*

TABLE 1. ^{13}C-nmr Data of Compounds **1–3** (20 MHz, C_5D_5N, TMS as internal standard).

Carbon	Compound		
	1	**2**[a]	**3**
C-1	35.8	35.8	34.1
C-2	26.8	22.1	26.5
C-3	74.8	78.0	74.4
C-4	38.4	38.6	38.0
C-5	49.4	49.0	46.0
C-6	19.2	19.3	18.8
C-7	36.0	35.8	32.4
C-8	42.7	42.4	43.8
C-9	55.9	52.7	152.2
C-10	39.8	38.8	39.2
C-11	69.8	72.3	116.0
C-12	38.4	34.9	37.0
C-13	37.5	36.9	38.0
C-14	42.9	42.8	40.4
C-15	29.9	29.9	30.1
C-16	32.8	32.3	32.5
C-17	56.5	56.3	56.0
C-18	47.5	46.6	47.7
C-19	49.4	49.8	49.0
C-20	151.0	150.0	150.8
C-21	31.0	30.8	29.8
C-22	37.5	36.9	37.0
C-23	29.6	29.5	28.6
C-24	22.7	21.9	22.5
C-25	18.0	17.8	22.0
C-26	17.4	17.2	22.0
C-27	14.4	14.5	16.3
C-28	179.2	180.9	178.7
C-29	110.0	110.4	110.3
C-30	19.2	19.3	19.3
*C*OOMe		170.5, 171.3	
COO*Me*		21.9, 21.3	

[a]In $CDCl_3$.

(rel. int.) $[M]^+$ 556 (3), $[M-HOAc]^+$ 496 (42), 437 (15), $[M-2HOAc]^+$ 436 (34), 421 (17), [a] 321 (12), [c] 285 (13), [h] 246 (20), [b] 234 (10), [b − H] 233 (26), [f] 220 (7), [f − H] 219 (34), 203 (26), 201 (26), 189 (39), 187 (32), 175 (36), 161 (18), 147 (39), 133 (62), 107 (100); ^{13}C nmr see Table 1.

DEHYDROIMPRESSIC ACID [3].—Impressic acid [1] (100 mg) was dissolved in 3 ml of Me_2CO and after addition of 5 drops of concentrated H_2SO_4, the solution was stirred at room temperature for 3 h. The solution was diluted with H_2O, extracted with $CHCl_3$, and dried over anhydrous Na_2SO_4. Distillation of the solvent under vacuum gave a product which on cc over Si gel gave the dehydrated product **3** (35 mg): mp 224–226° $[\alpha]^{25}D$ +1° (MeOH, $c=0.16$); ir ν max (KBr) 3441, 2960, 1705, 1642, 1618, 1465, 1388, 888, 759 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.80, 0.86, 0.88, 1.10, 1.20, (s, Me-23, -24, -25, -26, -27), 1.65 (s, Me-30), 3.35 (br s, $W_{1/2}=7$ Hz, H-3β), 4.57 and 4.72 (2xm, H_2-29), 5.22 (t, H-11); eims *m/z* (rel. int.) $[M]^+$ [d] 454 (23), $[M-H_2O]^+$ [e] 436 (90), 421 (100), $[M-H_2O-CO_2]^+$ 392 (22), [c] 285 (86), [h] 246 (32), [b] 234 (64), [a] 221 (34), [f] 220 (87), [f − H] 219 (57), 203 (84), 201 (53), 189 (86), 175 (74); ^{13}C nmr see Table 1.

3α-ACETOXYDEHYDROIMPRESSIC ACID [4].—A solution of **3** (10 mg) and Ac_2O/pyridine was allowed to stand at room temperature for 1 day. The crude product was passed through a small Si gel column to give **4** (8 mg): mp 190–192°; ir ν max (KBr) 1735 (ester), 1705 (COOH), 1645 (unsaturation), 1248 (OAc) cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.78, 0.83, 0.85, 1.08, 1.19 (s, Me-23, -24, -25, -26, -27), 1.65 (s, Me-30), 1.97 (s, OAc), 4.55 (brs, $W_{1/2}=7$ Hz, H-3β), 4.55 and 4.70 (2xm, H_2-29), 5.20 (t, H-11); eims *m/z* (rel. int.) $[M]^+$ [d] 496 (34), 437 (6), $[M-HOAc]^+$ [e] 436 (16), 421 (24), [c] 285 (35), [h] 246 (7), [b] 234 (5), [b − H] 233 (5), [f] 220 (7), [f − H] 219 (16), 189 (47), 187 (37), 175 (21), 161 (13), 133 (21), 121 (29), 107 (23), 55 (100), 43 (95).

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