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NEW SAPONINS FROM *MANGIFERA INDICA*

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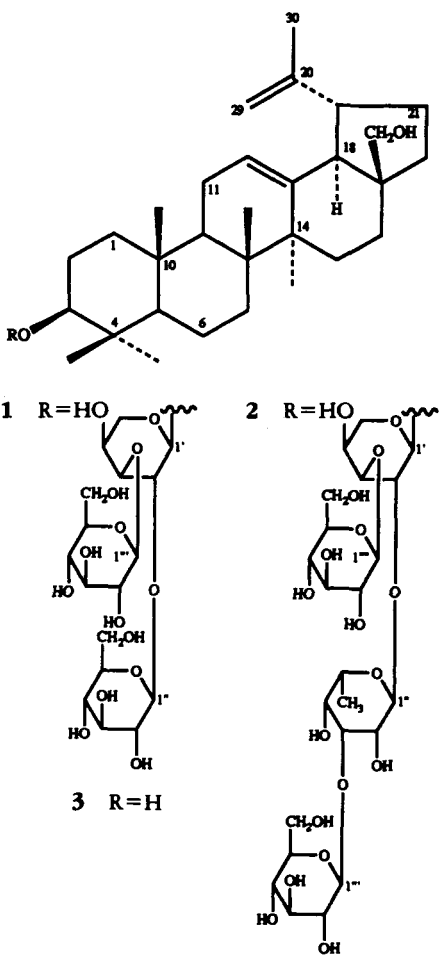
ABSTRACT.—Two new terpenoidal saponins, indicoside A [**1**] and indicoside B [**2**], were isolated from *Mangifera indica*. Their structures were determined as 28-hydroxylupa-12,20(29)-diene-3-*O*-[β-glucopyranosyl-(1↦2)][β-glucopyranosyl-(1↦3)]-α-L-arabinopyranoside [**1**] and 28-hydroxylupa-12,20(29)-diene-3-*O*-[β-glucopyranosyl-(1↦3)-α-L-rhamnopyranosyl-(1↦2)][β-glucopyranosyl(1↦3)]-α-L-arabinopyranoside [**2**] on the basis of chemical and spectroscopic studies.

Mangifera indica L. (Anacardiaceae) is widely distributed in the Indian subcontinent. Phytochemical studies on various parts of this plant have led to the isolation of a variety of flavonoids, steroids, and terpenoids (1–5). In this paper we report the isolation and structure elucidation of two new saponins, indicosides A [**1**] and B [**2**], from the air-dried stem bark of this plant.

RESULTS AND DISCUSSION

Indicoside A [**1**] was obtained in pure crystalline form by semipreparative hplc [RP-18 column, MeOH-H_2O (9:1)], mp 228–230°, $[\alpha]_D$ 67.35 ($c = 0.23$, MeOH). Compound **1** gave a positive Liebermann-Burchard test and a violet color with $Ce(SO_4)_2$. It also gave an apparent molecular ion peak in its negative fabms at *m/z* 895 $[M-H]^-$, consistent with the molecular formula $C_{47}H_{76}O_{16}$. Characteristic ions that appeared at *m/z* 733 $[M-H-162]^-$, 571 $[M-H-162-162]^-$, and 439 $[M-H-162-162-132]^-$ were generated by subsequent losses from the molecular ion of two hexose and one pentose units and clearly indicated that a hexose is the terminal sugar while a pentose is attached to the aglycone. The ir spectrum showed absorptions at 3450–3400 cm^{-1} (OH), 3045, 1650, 810 cm^{-1} (trisubstituted double bond), and 3070, 1640, 885 cm^{-1} ($C=CH_2$ group). The last set of absorption bands is characteristic of lupene derivatives (6) and suggested that the aglycone of **1** could belong to this class of triterpenes. The ^{1}H-nmr spectrum (CD_3OD, D_2O, 400 MHz) showed



three anomeric proton signals at δ 4.83 (d, J = 7.98 Hz), 4.59 (d, J = 7.2 Hz), 4.43 (d, J = 7.73 Hz), a terminal methylene group (m at δ 4.75), a methine proton (dd at δ 4.48, J = 10.13 Hz and 4.78 Hz), and six tertiary methyl groups (singlets at δ 1.67, 1.04, 0.98, 0.97, 0.96, and 0.83). A signal at δ 5.13 (br t, J = 3.58 Hz) was indicative of an olefinic proton attached to a trisubstituted double bond. Signals of a hydroxymethyl group were overlapped by the resonances of sugar moieties in the region 4.72–3.59 ppm. The ^{13}C-nmr spectrum showed 47 carbon atoms; the multiplicities of these were determined by using DEPT experiments (7,8), which revealed the presence of 6 methyl, 14 methylene, and 20 methine carbon atoms. Of these, 30 carbon signals were due to the aglycone moiety, and the signals at δ 106.01, 105.03, and 103.72 indicated anomeric carbon atoms and confirmed that the carbohydrate chain was composed of three sugar units.

Acid hydrolysis of **1** yielded sapogenin **3**, glucose, and arabinose, which were identified by tlc and by comparison with authentic sugar samples. Compound **3** was purified through preparative tlc and its eims revealed fragment ions at *m/z* 203, 201, 189, and 187 which are characteristic of 12,13-dehydrolupenes (9). These results, along with comparison of the ^{1}H- and ^{13}C-nmr data of **1** with those of related lupene derivatives (10–12) suggested that the aglycone moiety in indicoside A was lupa-12,20(29)-diene-3β-28-diol. Further, the downfield shift of C-3 (aglycone) indicated sugar moiety attachment at this position. The anomeric configurations of sugar were deduced by coupling constants in the ^{1}H-nmr spectrum of **1** which showed β configurations for D-glucose units and α for L-arabinose. The sugar linkages and sequencing were determined through ^{13}C nmr and negative fabms, respectively. The ^{13}C-nmr assignments of two β-D-glucose units were identical with those of the corresponding methyl glucoside, indicating that they were terminal sugars. The downfield chemical shifts of C-2 and C-3 of arabinose, which appeared at δ 78.17 and 84.37, represented (1↦2) and (1↦3) linkages of glucose units to arabinose (13,14). Thus, the structure of indicoside A was assigned as **1**.

Indicoside B [**2**] was eluted from the same column [MeOH-H_2O (8.5:1.5)]; mp 242–244°, [α]D 63.18 (c = 0.39, MeOH). Compound **2** gave an apparent molecular ion peak at *m/z* 1041 $[M-H]^-$ (pseudo-molecular ion) consistent with the molecular formula $C_{53}H_{86}O_{20}$. The ions at *m/z* 879 $[M-H-162]^-$, 717 $[M-H-162-162]^-$, 571 $[M-H-162-162-146]^-$, and 439 $[M-H-162-162-146-132]^-$ represented losses of two hexose, one deoxyhexose, and one pentose unit from the molecular ion. The ir spectrum showed bands at 3445–3400 cm^{-1} (OH groups), 3050, 1650, 810 cm^{-1} (trisubstituted double bond), and 3070, 1645, 885 ($C=CH_2$ group). The ^{1}H nmr spectrum [CD_3OD-D_2O (1:1), 400 MHz] showed four anomeric proton signals at δ 4.53 (d, J = 7.71 Hz), 5.12 (d, J = 1.54 Hz), 4.42 (d, J = 7.50 Hz), 4.32 (d, J = 7.61 Hz), an olefinic proton (br t at δ 5.14, J = 3.62 Hz), a terminal methylene group (m at δ 4.73), a methine proton (dd at δ 4.47, J = 10.78 Hz and 4.63), and six tertiary methyl groups (singlets at δ 1.66, 1.04, 0.98, 0.97, 0.95, and 0.83). The ^{13}C-nmr spectrum showed 53 carbon atoms; their multiplicities were determined by DEPT experiments (7,8), which showed 7 methyl, 14 methylene, and 25 methine carbon atoms. Out of these, 30 were due to the aglycone moiety and were identical to the ^{13}C-nmr chemical shifts of the aglycone of indicoside A [**1**]. Hence the aglycone in both **1** and **2** was identical. The downfield shift of C-3 of its aglycone again indicated attachment of the sugar moiety at this position. The carbohydrate chain was comprised of four sugar units, confirmed by

anomeric carbons at δ 105.01, 101.87, 104.07, and 104.96. These were identified as one arabinose, one rhamnose, and two glucose units by acid hydrolysis of **2** and subsequent tlc comparison with authentic sugar samples. The anomeric configurations of these were deduced by coupling constants which indicated its β configuration for D-glucose and α for L-arabinose and L-rhamnose. The ^{13}C-nmr assignments of two β-D-glucose units were identical with corresponding methyl glycoside (13,14) indicating that they were terminal sugars. The downfield chemical shifts of C-2 and C-3 of arabinose which appeared at δ 76.69 and 84.34 represented (1↦2) and (1↦3) linkages of rhamnose and glucose units to arabinose. On the other hand the downfield shift of the C-3 of rhamnose, which appeared at δ 83.01, suggested a (1↦3) linkage of glucose to rhamnose. Thus indicoside B was concluded to have structure **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded on a JASCO A-302 spectrometer. The negative fabms were recorded on a Finnigan MAT-312 spectrometr connected to a PDP 11/34 (DEC) computer system. The ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-400 spectrometer in CD_3OD-D_2O (1:1) with TMS as internal reference. The DEPT experiments were carried out with the last pulse angle t = 45°, 90°, and 135°. The quaternary carbons were determined by subtraction of these spectra from the broad-band ^{13}C-nmr spectrum.

PLANT MATERIAL.—The plant material was collected in Karachi, Pakistan and was identified by Professor Dr. Mohammad Qaiser, Department of Botany, University of Karachi (voucher No. KUH 4378).

ISOLATION OF THE COMPOUNDS.—The air-dried stem bark of *M. indica* (10 kg) was ground and extracted thrice with MeOH (40 liters). The combined MeOH extract was evaporated under reduced pressure, and the resulting residue was partitioned with EtOAc and H_2O. The H_2O layer was extracted with *n*-BuOH and on evaporation afforded a gummy mixture of saponins (3.87 g). This on cc over Si gel (150 g) with $CHCl_3$-MeOH (1:1) yielded a fraction (135 mg) which was purified by hplc on an RP-18 column solvent system MeOH-H_2O (9:1 and 8.5:1.5), to afford indicoside A [**1**] (16 mg) and indicoside B [**2**] (12 mg).

Indicoside A [**1**].—Compound **1** crystallized from aqueous MeOH: mp 228–230°; [α]D 67.35 (*c* = 0.23, MeOH); ir (KBr) ν max cm^{-1} 3450–3400, 3070, 3045, 1650, 1640, 885, 810; negative fabms *m/z* 895, 733, 571, 439; ^{1}H nmr [CD_3OD-D_2O (1:1)] (400 MHz) δ 5.13 (1H, br t, *J* = 3.58 Hz, H-12), 4.83 (1H, d, *J* = 7.98 Hz, H-1′), 4.75 (2H, m, H_2-29), 4.59 (1H, d, *J* = 7.28 Hz, H-1″), 4.48 (1H, dd, *J* = 10.13 Hz and 4.78 Hz, H-3), 4.43 (1H, d, *J* = 7.37 Hz, H-1‴), 4.72–3.59 (overlapped signals of hydroxy groups), 1.67 (3H, s, Me-30), 1.04 (3H, s, Me-23), 0.98 (3H, s, Me-26), 0.97 (3H, s, Me-27), 0.96 (3H, s, Me-24), 0.83 (3H, s, Me-25); ^{13}C nmr (CD_3OD + D_2O, 100.61 MHz) δ 39.97 (C-1), 24.21 (C-2), 87.98 (C-3), 39.74 (C-4), 56.28 (C-25), 18.64 (C-6), 35.89 (C-7), 42.09 (C-8), 52.01 (C-9), 37.65 (C-10), 22.63 (C-11), 125.46 (C-12), 138.97 (C-13), 42.79 (C-14), 27.61 (C-15), 34.63 (C-16), 47.81 (C-17), 49.68 (C-18), 47.91 (C-19), 151.57 (C-20), 30.97 (C-21), 37.68 (C-22), 28.07 (C-23), 16.73 (C-24), 16.17 (C-25), 16.25 (C-26), 14.87 (C-27), 60.97 (C-28), 109.49 (C-29), 19.47 (C-20), 106.01 (C-1′), 78.17 (C-2′), 84.37 (C-3′), 69.71 (C-4′), 66.53 (C-5′), 103.72 (C-1″), 73.83 (C-2″), 77.84 (C-3″), 70.11 (C-4″), 76.05 (C-5″), 62.24 (C-6″), 105.03 (C-1‴), 73.81 (C-2‴), 77.83 (C-3‴), 70.11 (C-4‴), 76.17 (C-5‴), 62.24 (C-6‴). Assignments were made through comparison with published ^{1}H- and ^{13}C-nmr spectral data of related compounds (10–14).

Acid hydrolysis of **1**.—Compound **1** (10 mg) in aqueous MeOH (4.0 ml) was hydrolyzed with 10% HCl (2.0 ml) on a boiling H_2O bath for 4.0 h. The MeOH was evaporated under reduced pressure. The mixture was diluted with H_2O, extracted with EtOAc, dried over anhydrous Na_2SO_4 and evaporated to yield, after preparative tlc, **3** as a colorless oil (4.73 mg): ms *m/z* (rel. int. %) [M]$^{+}$ 440 (24), 425 (11), 203 (11), 201 (8), 189 (12), 187 (16); ^{1}H nmr [$CDCl_3$-D_2O (1:1)] 5.12 (1H, brt, *J* = 3.58 Hz, H-12), 4.48 (1H, dd, *J* = 10.13 Hz and 4.78 Hz, H-3), 1.66 (3H, s, Me-30), 1.04 (3H, s, Me-23), 0.98 (3H, s, Me-26), 0.96 (3H, s, Me-27), 0.96 (3H, s, Me-24), 0.83 (3H, s, Me-25), 4.74 (2H, m, H_2-29).

Indicoside B [**2**].—Compound **2** recrystallized with aqueous MeOH: mp 242–244°; [α]D 63.18 (*c* = 0.34, MeOH); ir (KBr) ν max cm^{-1} 3445–3400, 3070, 3050, 1650, 1645, 885, 810; negative fabms *m/z* 1041, 879, 717, 571, 439; ^{1}H nmr [CD_3OD-D_2O (1:1)] (400 MHz) δ 5.14 (1H, br t, *J* = 3.62 Hz, H-12), 5.12 (1H, d, *J* = 1.54 Hz, H-1″), 4.73 (2H, m, H_2-29), 4.53 (1H, d, *J* = 7.71 Hz, H-1′), 4.47 (1H, dd, *J* = 10.78 Hz and 4.63 Hz, H-3), 4.42 (1H, d,

J = 7.50 Hz, H-1‴), 4.32 (1H, d, J = 7.61 Hz, H-1⁗), 1.66 (3H, s, Me-30), 1.04 (3H, s, Me-23), 0.98 (3H, s, Me-26), 0.97 (3H, s, Me-27), 0.95 (3H, s, Me-24), 0.83 (3H, s, Me-25); ^{13}C nmr ($CD_3OD + D_2O$, 100.61 MHz) δ 39.97 (C-1), 24.21 (C-2), 87.96 (C-3), 39.75 (C-4), 56.28 (C-5), 18.64 (C-6), 35.89 (C-7), 42.10 (C-8), 52.01 (C-9), 37.65 (C-10), 22.63 (C-11), 125.48 (C-12), 138.98 (C-13), 42.79 (C-14), 27.61 (C-15), 34.63 (C-16), 47.81 (C-17), 49.68 (C-18), 47.91 (C-19), 151.58 (C-20), 30.97 (C-21), 37.68 (C-22), 28.06 (C-23), 16.75 (C-24), 16.17 (C-25), 16.24 (C-26), 14.87 (C-27), 60.93 (C-28), 109.48 (C-29), 19.48 (C-30), 105.01 (C-1′), 76.69 (C-2′), 84.34 (C-3′), 68.41 (C-4′), 64.67 (C-5′), 101.87 (C-1″), 72.19 (C-2″), 83.01 (C-3″), 74.01 (C-4″), 70.13 (C-5″), 17.96 (C-6″), 104.07 (C-1‴), 74.97 (C-2‴), 78.13 (C-3‴), 70.21 (C-4‴), 76.98 (C-5‴), 62.43 (C-6‴), 104.96 (C-1⁗), 74.96 (C-2⁗), 78.16 (C-3⁗), 70.21 (C-4⁗), 76.51 (C-5⁗), 62.44 (C-6⁗).

Acid hydrolysis of **2**.—Compound **2** (10 mg) in aqueous MeOH (4.0 ml) was hydrolyzed with 10% HCl (2.0 ml) on a boiling H_2O bath for 4.0 h. The reaction mixture was worked up in the usual manner to afford **3** as a colorless oil (3.88 mg): ms *m/z* (rel. int. %) $[M]^+$ 440 (28), 425 (13), 203 (12), 201 (10), 189 (17), 187 (19).

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