TRITERPENOIDS OF *PERIPLOCA CALOPHYLLA*

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ABSTRACT.—Three new triterpenoid acids of the oleanane series (P_1, P_2 and P_3) have been isolated from the twigs of *Periploca calophylla*. They have been characterized on the basis of chemical and spectroscopic evidences.

The plants belonging to the Asclepiadaceae family are rich in cardiac and pregnane glycosides (1,2). In an earlier chemical investigation of the twigs of *Periploca calophylla*, the presence of periplogenin and cymarose had been reported (3). In a recent reinvestigation of the dried twigs of this plant, the crude genin and glycoside mixtures were obtained, which, on preparative isolation, afforded a new pregnane glycoside, calocin (4), and a cardiac glycoside, phyllacin (5). These extracts also afforded β-amyrin, α-amyrin acetate, and small quantities of three hitherto unreported triterpenoids designated as compounds P_1, P_2, and P_3. The characterization of these compounds is now reported.

RESULTS AND DISCUSSIONS

COMPOUND P_1.—The molecular formula $C_{30}H_{48}O_3$ of compound P_1, mp, 219-221°, $[\alpha]_D+52$, was in agreement with its M^+ at *m/e* 456. In its ms, the characteristic peaks at *m/e* 248 (100%) and 203 (36.8%, 248-COOH) due to retro-Diels-Alder fragmentation, indicated that it was a derivative of an olean-12-en or urs-12-en-28-carboxylic acid having no other substituent in C, D, and E rings (6,7,8). Its pmr spectrum displayed appropriate signals for seven tertiary C-methyl groups, a vinyl proton, and a secondary carbinol methine proton.

Acetylation of P_1 furnished a monoacetate, $C_{32}H_{50}O_4$ (M^+, *m/e* 498), mp 210-212°, $[\alpha]_D+45$. Its pmr spectrum exhibited, besides other signals, a 1H multiplet characteristic of a C-18 proton of an olean-12-en system (9) and an acetyl singlet. The 1H triplet attributed to the carbinol methine proton in the pmr of P_1, on acetylation, had suffered a downfield shift by 1.32 ppm, thus confirming the assignment. However, the mp and rotation of P_1 and those of its acetate were not identical to those of the known 3-hydroxy olean-12-en-28-carboxylic acid, oleanolic acid (10) (mp, 305-10°, $[\alpha]_D+79.5$), its acetyl derivative (mp 268°, $[\alpha]_D+72.8$) or epi- oleanolic acid (11) (mp, 297-298°, $[\alpha]_D+68$ and its acetyl derivative (mp, 265-267°, $[\alpha]_D+29$), as well as to the new monohydroxy olean-12-en-28-carboxylic acid (12) (mp, 247-249°). Compound P_1 is, thus, a hitherto unreported monohydroxy olean-12-en-28-carboxylic acid.

COMPOUND P_2.—This compound, $C_{30}H_{48}O_4$, mp, 235-240°, $[\alpha]_D+54$ showed the molecular ion at *m/e* 472 in ms, which is in agreement with the molecular formula. Its prominent ions at *m/e* 248 (100%), 203 (61.4%, 248-COOH) due to retro-Diels-Alder fragmentation were again characteristic of an olean-12-en or urs-12-en-28-carboxylic acid series suggesting the absence of any substituent group in rings C, D, and E (6-8). The two oxygen functions of this compound were, therefore, present in ring A and/or B. The pmr spectum of P_2 contained signals for six tertiary C-methyl groups, a CH_2-O-, and a CH- O-.

Acetylation of P_2 afforded a diacetate $C_{34}H_{52}O_6$, mp, 92-95°; its pmr spectrum also had signals for six tertiary C-methyl groups, two acetyl groups, a 1H multiplet characteristic of a C-18 methine proton, a tertiary C-CH_2OAc, a CHOAc, and a vinyl

proton, which was unavoidably masked in the pmr of P_2 by the traces of moisture in pyridine-d5. The position and multiplicity of the C*H*OH proton signal demanded its placement at C-3. Assuming the presence of a 3β-secondary hydroxyl group, the primary hydroxyl group could be present at either C-23 or C-24. The pmr spectrum of tri-O-acetyl P_2 indicated the position of the primary hydroxyl group at C-23, because the position of the AB spectra (centered at δ 3.69 ppm) is in full agreement with the reported position for a 4-equatorical C*H*$_2$OAc group (13).

When P_2 was shaken with acetone in the presence of anhydrous $CuSO_4$ (14), it furnished a compound of higher mobility on tlc, which was presumably an acetonide derivative. If it is so, then the secondary and primary hydroxyl must be present at C-3β and C-23 positions, respectively. Treatment of P_2 acetate with diazomethane gave an amorphous methyl ester displaying its molecular ion at *m/e* 570 in the ms, in full agreement with the expected molecular formula $C_{35}H_{54}O_6$. The pmr spectrum of methyl ester acetate also contained signals for six tertiary C-methyl groups, two acetyl groups, a C-18 methine proton, a -COOC*H*$_3$, a C*H*$_2$OAc, a C*H*-OAc, and a vinyl proton. A comparison of mp and rotation of P_2 and its acetate showed it to be different from the known dihydroxy olean-12-en-28-carboxylic acid, hederagenin (15) (mp. 333-34°, [α]D+82] and hederagenin diacetate (mp, 168-70°, [α]D+66.2).

COMPOUND P_3.—This compound, $C_{30}H_{48}O_5$, (M^+, 488), mp, 225-227°, [α]D+18.6, also showed prominent ms peaks at *m/e* 248 (100%) and *m/e* 203 (86.5%, 248-COOH), suggesting it to be an olean-12-en or urs-12-en-28-carboxylic acid without any substituent in C, D, and E rings. Its acetylation afforded a triacetate, mp, 95-100°. Treatment of the latter with diazomethane yielded an amorphous mono-methyl ester of the triacetate, indicating that the compound P_3 contained three hydroxyl groups and a carboxylic group, which accounted for all five oxygen atoms in the molecule. The positive $NaIO_4$ reaction (16) of P_3 suggested it to contain a vicinal diol system in the molecule.

The pmr spectrum of P_3-triacetate showed it to contain six tertiary C-methyl groups, three acetyl groups, a tertiary -C*H*$_2$OAc, a vinyl proton signal, and acetoxy methine protons. The pmr spectrum of methyl ester triacetate was more informative, as it clearly showed the characteristic C-18 olean methine proton. It also contained signals for six tertiary C-methyl groups, three acetyl groups, a -COOC*H*$_3$, a tertiary C*H*$_2$OAc. Because compound P_3 gave rise to two components of higher Rf on tlc with acetone in presence of $CuSO_4$ (anhydrous), presumably isopropylidene derivatives, this suggests that C-3 and C-23 hydroxyl groups are present and the third hydroxy group must, therefore, be present at C-2 to substantiate its vicinal position responsible for the $NaIO_4$ reaction exhibited by P_3. Compound P_3 was, therefore, 2,3,23-trihydroxy olean-12-en-28-carboxylic acid. Rotation and mp of P_3, however, was different from that of arjunolic acid (17, 18) (mp 337-340°, [α]D+63.5). The unknown compound P_3 is, thus, not identical with the known 2,3,23-trihydroxy olean-12-en-28-carboxylic acid nor with the new trihydroxy olean-12-en-28-carboxylic acid reported by Y. Yazaki (12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All the melting points were determined on a Boetius micro melting point apparatus and are uncorrected. Optical rotations were measured in a 1-dm tube with a Jasco-Dip 180 automatic polarimeter. The ir spectra were recorded on a Perkin-Elmer IR-177 spectrophotometer and the pmr spectra on a 90-MHz Perkin-Elmer R-32 in $CDCL_3$ (unless otherwise mentioned), with M_4Si as the internal standard. Mass spectra were recorded on a JEOL JMS 300 mass spectrometer. The tlc plates were run on silica gel G layers and columns on silica gel (BDH) and alumina (E. Merck).

Shade-dried twigs (5 kg) of *Periploca calophylla* were extracted and fractionated with solvents of differ-

ent polarities, as reported earlier (4), to afford petroleum ether extract (2.5 g), ether extract (1.0 g), chloroform extract (20 g). chloroform-ethanol extract 4:1 (7 g), and chloroform-ethanol extract 3:2 (2.5 g), respectively. Combined ether and chloroform extracts (21 g) were chromatographed on alumina (400 g), by collection of 250-ml fractions. Fractions 1-6. eluted with benzene and repeatedly chromatographed over silica gel, afforded α-amyrin acetate (450 mg), mp, 202-205°, $[\alpha]_D$+70 (c = 0.5, ethanol), β-amyrin (100 mg) mp, 188-190°, $[\alpha]_D$+75 (c =0.9, ethanol); fractions 25-31, eluted with chloroform, afforded P_1 (150 mg); fractions 32-35, eluted with chloroform-methanol 98:2, afforded P_2 (110 mg); fractions 36-42, eluted with chloroform-methanol 94:6, afforded P_3 (50 mg).

Substance P_1.—It crystallized from ethanol as colorless prisms, mp, 219-21°, $[\alpha]_D$+52 (c = 0.57, ethanol). It gave positive Liebermann-Burchardt test (19-20) and effervescence with $NaHCO_3$ for a carboxylic group. It showed νmax (KBr) cm^{-1}: 3400-3200, 2900, 1685, 1460, 1380, 1280, 1033, 1000, and 838; pmr data: δ 5.16 (m, 1H, H-12), 3.1 (t, 1H, J=8Hz, H-3), 1.08 (s, 1×CH_3), 0.90 (s, 1×CH_3), 0.80 (s, 3×CH_3), 0.72 (s, 1×CH_3) and 0.70 (s, 1×CH_3); ms: *m/e* 456 (4.2%, M^+), 438 (1.2), 410 (0.8), 300 (1.8), 249 (18.4), 248 (100), 233 (2.3), 219 (5.8), 207 (23.1), 203 (36.8), 190 (9.2), 173 (2.1), 147 (4.9), 145 (2.1), 133 (21.3), 119 (8.3), 95 (6.8), 83 (10.6), 55 (10.4), and 43 (17.7).

Anal. calcd for $C_{30}H_{48}O_3.H_2O$: C, 75.95, H. 10.55. Found C, 75.76; H. 10.48.

Mono-O-acetyl P_1.—A solution of P_1 (20 mg) in pyridine (0.4 ml) and acetic anhydride (0.4 ml) was kept for 48 h at room temperature. The pyridine and excess of acetic anhydride were then removed under reduced pressure. The viscous residue taken in chloroform, was washed in sequence with 2 N HCl, 2 N Na_2CO_3 solution, and water, then dried over Na_2SO_4, filtered, and evaporated to yield an acetylated product (20 mg), which crystallized from methanol mp, 210-12°, $[\alpha]_D$+45 (c =0.92, chloroform), pmr data; δ 5.15 (m, 1H, H-12), 4.42 (t, 1H, J = 8 Hz, H-3), 2.65 (m, 1H, H-18), 1.97 (s, 3H, 1 × Ac), 1.01 (s, 1 × CH_3), 0.91 (s, 1 × CH), 0.80 (s, 1 × CH_3), 0.71 (s, 3 × CH_3), 0.70 (s, 1 × CH_3); ms: *m/e* 498 (2%, M^+), 452 (1.1), 438 (7.0), 423 (2.9), 395 (1.8), 327 (1.0), 315 (0.79), 287 (1.8), 262 (2.1), 257 (2.1), 249 (68), 241 (2.0), 235 (10.4), 219 (15.4), 200 (30.0), 191 (27.9), 190 (67.0), 189 (46.5), 175 (15.6), 147 (17.1), 133 (15.2), 199 (27.1), 109 (13.4), 107 (18.6), 105 (10.2), 55 (25.8), and 48 (38.8).

Anal. calcd for $C_{32}H_{50}O_4$: C, 77.11; H, 10.0. Found: C, 77.06; H. 9.99.

Substance P_2.—It crystallized from ethanol as colorless needles, mp, 235-40°, $[\alpha]_D$+54, (c = 0.90, ethanol). It showed νmax (KBr) cm^{-1}: 3440, 2940, 1692, 1445, 1382, 1230, 1185, 1048, 980, and 825; pmr data: δ 3.69 and 3.73 (2s, 1H each, H-23 & H′-23), 3.18 (m, 1H, H-18), 1.18 (s, 1×CH_3), 1.06 (s, 2×CH_3), 1.03 (s, 2×CH_3), 0.98 (s, 1×CH_3); ms: *m/e* 472 (1.2%, M^+), 454 (0.9), 436 (0.7), 426 (1.1), 408 (0.8), 302 (0.8), 248 (100), 233 (5.1), 219 (8.8), 203 (61.4), 189 (10.2), 175 (10.9), 173 (5.9), 147 (7.5), 145 (4.8), 133 (34.3), 55 (10.4), 43 (9.5), and 18 (15.6).

Anal. calcd for $C_{30}H_{48}O_4$: C, 76.27; H. 10.17. Found C, 76.02; H, 9.99.

Di-O-acetyl P_2.—Crystalline P_2 (15 mg), dissolved in anhydrous pyridine (0.3 ml), was mixed with acetic anhydride (0.3 ml); the mixture was kept for 48 h at room temperature. After the usual work-up of the reaction mixture as for P_1, it afforded an acetylated product as an amorphous residue that failed to crystallize. The pmr data: δ 5.17 (m, 1H, H-12), 4.7 (q, 1H, J = 8 and 3.5 Hz, H-3), 3.58 and 3.80 (2d, 1H each, J = 12 Hz, H-23 & H′-23), 2.68 (m, 1H, H-18), 1.20 (s, 2×CH_3), 1.01 (s, 1×CH_3), 0.84 (s, 2×CH_3), 0.78 (s, 1×CH_3); ms: *m/e* 496 (2.8%, M-60), 450 (1.4), 436 (4.4), 421 (1.9), 393 (1.2), 383 (1.1), 369 (1.5), 357 (1.2), 335 (1.6), 307 (6.5), 262 (11.7), 249 (24.7), 248 (100), 235 (7.6), 219 (9.4), 203 (43.2), 189 (26.9), 175 (10.9), 173 (11.9), 159 (10.2), 147 (13.0), 145 (11.4), 133 (43.1), 121 (15.7), 112 (10.8), 109 (18.8), 95 (25.7), 69 (28.8), and 55 (35.5).

Methyl ester of P_2 *acetate.*—The substance (10 mg) was dissolved in methanol (0.2 ml) and treated with an ether solution of diazomethane. Next day, the ether was removed to get the methylated product, which failed to crystallize. The pmr data: δ 5.11 (m, 1H, H-12), 4.59 (t, 1H, J=8Hz, H-3), 3.50 and 3.65 (2s, 1H each, H-23 & H′-23) 3.48 (s, 3H, $COOCH_3$), 1.90 and 1.97 (2s, 3H each, 2×Ac), 1.12 (s, 2×CH_3), 1.02 (s, 1×CH_3), 0.93 (s, 1×CH_3), 0.90 (s, 1×CH_3), 0.78 (s, 1×CH_3); ms: *m/e* 570 (4.7%, M^+) 510 (6.3), 450 (3.0), 435 (1.5), 407 (0.50), 391 (1.44), 375 (0.5), 355 (0.4), 317 (1.3), 307 (2.8), 298 (2.1), 262 (100), 249 (6.0), 233 (3.8), 223 (3.0), 217 (1.3), 203 (81.7), 189 (22.5), 167 (13.5), 147 (17.0), 133 (32.1), 119 (17.9), 108 (12.1), 95 (15.8), 87 (15.6), 81 (16.2), 75 (12.7), 69 (20.4), 55 (28.3), and 46 (65.1).

Isopropylidene derivative of P_2.—A solution of P_2 (5 mg) in dry acetone (5 ml) was shaken with anhydrous $CuSO_4$ (25 mg) at 25° for 10 days. It showed a new spot on tlc, indicating the possible formation of an isopropylidene derivative.

Substance P_3.—It crystallized from ethanol as colorless needles, mp, 225-27°, $[\alpha]_D$+18.6. It showed positive Liebermann-Burchardt test and effervescence with $NaHCO_3$ for a carboxylic group. It showed νmax (KBr) cm^{-1}: 3460-3400, 2918, 2860, 1695, 1460, 1385, 1308, 1235, 1130, 1055, 800, 770, and 660; ms: *m/e* 488 (0.4%, M^+), 457 (0.6), 452 (10.7), 437 (0.7), 355 (0.6), 339 (1.2), 316 (0.9), 301 (1.2), 262 (1.1), 248 (100), 235 (5.1), 219 (9.1), 203 (86.5), 191 (15.6), 189 (14.5), 173 (14.7), 147 (10.5), 133 (50.3), 121 (13.6), 119 (23.5), 107 (15.3), 95 (23.0), 81 (19.5), 69 (22.8), 55 (23.6), 43 (20.1), 28 (10.3), and 18 (34.9).

Anal. calcd for $C_{30}H_{48}O_5$: C, 75.82; H, 9.82. Found C, 75.61; H, 9.81.

Periodate oxidation of P_3.—To a solution of crystalline P_3 (2 mg) in methanol (0.2 ml) was added a solution of sodium metaperiodate (6 mg) in water (0.1 ml). The mixture was kept for 4 h at room temperature, diluted with water (0.4 ml), and evaporated under reduced pressure. The residue showed complete consumption of P_3 by co-chromatography in tlc chloroform-methanol 9:1.

Tri-O-acetyl P_3.—Crystalline P_3 (20 mg) dissolved in anhydrous pyridine (0.4 ml) was mixed with acetic anhydride (0.4 ml). This mixture was kept for 48 h at room temperature. After the usual work-up of the reaction mixture as for P_1, it afforded an acetylated product that crystallized from methanol as colorless needles, mp, 95-97°. It showed νmax (KBr) cm^{-1}: 3400, 2900, 1738, 1442, 1360, 1225, and 1040; pmr data: δ 5.15 (m, 1H, H-12), 5.0 (m, 2H, H-2 and H-3), 3.80, and 3.50 (2d, 1H each J = 12 Hz, H-23 and H′-23), 1.91, 1.95, and 2.01 (3s, 3H each, 2×Ac), 1.20 (s, 3×CH_3), 1.08 (s, 1×CH_3), 0.83 (s, 2×CH_3).

Anal. calcd for $C_{36}H_{54}O_8$: C, 70.35; H, 8.79. Found C, 70.32; H, 8.75.

Methyl ester of P_3 *acetate*.—The substance (10 mg) was dissolved in methanol and treated with an ether solution of diazomethane. Next day, the ether was removed to afford the methylated product, which failed to crystallize; pmr data: δ 5.20 (m, 2H, H-2 and H-3), 5.1 (m, 1H, H-12), 3.5 and 3.6 (2s, 1H each, H-23 and H′-23), 3.48 (s, 3H, $COOCH_3$), 1.83, 1.88, 1.98 (3s, 3H each, 3×Ac), 1.09 (s, 3×CH_3), 1.03 (s, 1×CH_3), 0.81 (s, 1×CH_3), 0.80 (s, 1×CH_3).

Isopropylidene derivative of P_3.—A solution of P_3 (5 mg) in dry acetone (5 ml) was shaken with anhydrous $CuSO_4$ (25 mg) at 25° for 10 days. It showed two new spots on tlc, indicating the formation of two isopropylidene derivatives.

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