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CYCLOARTANE-TYPE TRITERPENES FROM *AMBERBOA RAMOSA*

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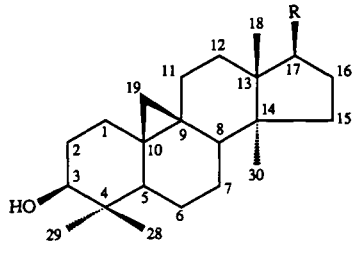
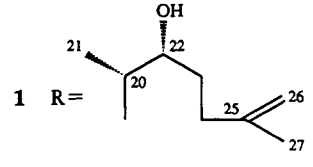
ABSTRACT.—Two new triterpenes have been isolated from *Amberboa ramosa*. Their structures were established as (22*R*)-cycloart-25-ene-3β,22-diol [**1**] and cycloart-20-ene-3β,25-diol [**2**] through chemical and spectroscopic studies including 2D nmr. Two known triterpenes, cycloartanol and cycloart-23-ene-3β,25-diol, were also isolated.

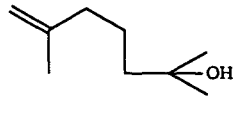
Several species of *Amberboa* (Compositae) have been reported to possess cytotoxic and antibacterial activity (1–3). One of these species, *Amberboa ramosa* Jafri (syn. *Vollutarella divaricata*) is found in India and Pakistan. It is reported to have tonic, aperient, febrifuge, and deobstruent properties (4,5). An EtOH extract of this plant was also found to possess cytotoxic activity (6). Previous chemical study on *A. ramosa* resulted in isolation of known flavonoids, steroids, and steroidal glycosides (6). Further study on the fresh and undried plant material has now resulted in the isolation of two new cycloartane-type triterpenes, (22*R*)-cycloart-25-ene-3β,22-diol [**1**] and cycloart-20-ene-3β,25-diol [**2**]. The known triterpenes cycloartanol and cycloart-23-ene-3β,25-diol have also been isolated from this species for the first time.

RESULTS AND DISCUSSION

Compound **1**, mp 180–181°, $[\alpha]^{20}D$ +20.6° ($CHCl_3$), was consistent with molecular formula $C_{30}H_{50}O_2$ by hrms ($[M]^+$ *m/z* 442.3824, calcd 442.3980) indicating six double bond equivalents in the molecule. It gave positive Liebermann-Burchard and ceric sulfate tests for triterpenes. The ir spectrum of **1** showed the presence of hydroxyl groups (3600–3450 cm^{-1}), a cyclopropane ring (3045 cm^{-1}), and a terminal methylene group (1650 and 890 cm^{-1}). The ^1H-nmr (500 MHz) spectrum showed signals due to five tertiary methyl groups (δ 0.80, 0.88, 0.95, 0.96, and 1.60) and one secondary methyl group (δ 0.87, d, $J = 6.8$ Hz). A pair of doublets at δ 0.31 and 0.54 ($J = 4.2$ Hz) was indicative of a cyclopropane ring bearing two nonequivalent hydrogen atoms. The double







doublet at δ 3.20 and doublet of double doublets at δ 4.06 were due to the protons attached to the carbons bearing hydroxyl groups, while the broad singlets at δ 4.80 and 4.49 (1H each) could be assigned to a vinylidene group. The ^{13}C-nmr spectrum showed 30 carbon atoms. The multiplicity assignments were made by DEPT experiments, which revealed the presence of six methyl, twelve methylene, six methine, and six quaternary carbon atoms.

The presence of two secondary hydroxyl groups was confirmed by oxidation of **1** to a diketone. The diketone gave a positive Zimmermann test (7), indicating the presence of one oxo group at position 3. Further insight into the structure of **1** was obtained from the mass spectrum. Compound **1** showed a daughter ion at *m/z* 355.3004 ($C_{25}H_{39}O$) corresponding to the elimination of C_5H_9 from $[M-18]^+$, which is characteristic of 4,4-dimethyl-9:19-cyclosterol (8). Another characteristic process involved elimination of ring A (8,9), producing an ion at *m/z* 302.2621 ($C_{21}H_{34}O$) and another peak at *m/z* 175.1465 ($C_{13}H_{19}$) from the loss of the $C_8H_{15}O$ side chain from this *m/z* 302 parent ion. The presence of a monounsaturated side chain was also evident by the peaks at *m/z* 315.2682 ($C_{22}H_{35}O$), 313.2654 ($C_{22}H_{33}O$), and 297.2562 ($C_{22}H_{33}$) (8). All of these fragments were consistent with the presence of one of the secondary hydroxyl functions in the side chain.

As the $[M-side\ chain-2H]^+$ ion was very weak, the side chain unsaturation should be distant from the nucleus (9). A double bond at C-25 was indicated by absence of the characteristic 6H doublet of an isopropyl group and the presence of a vinyl methyl singlet at δ 1.62. The elimination of part of the side chain in a McLafferty process (10) was also apparent, especially in the spectrum of the diketone of **1** $[M-56]^+$.

Five protons were exchangeable with D_2O in base in the ^{1}H-nmr spectrum of the diketone of **1**. In a ^{1}H-^{1}H homonuclear chemical shift correlation spectrum (COSY-45) of **1** the comparatively upfield carbinol methine proton at δ 3.20 showed cross peaks with two other protons and hence could be assigned to C-3. The large coupling ($J_{ax,ax}=9.8$ and $J_{ax,eq}=4.11$ Hz) allowed us to assign β and equatorial orientation to the hydroxyl group at C-3. On the other hand, the downfield proton at δ 4.06 showed connectivity with three other protons, limiting the position of the corresponding hydroxyl group to C-22. The doublet at δ 0.87 was assigned to H_3-21, showing a cross peak with H-20 at δ 1.43. The H-22 at δ 4.06 showed cross peaks with both the protons at C-23 ($J=7.61$ and 5.2 Hz) as well as weak coupling (1 Hz) with H-20 (observable in the 2D *J*-resolved spectrum), which agrees with that reported in the literature (11) as the angle is near 90°. Spin decoupling combined with nOe difference spectroscopy allowed complete assignment of the stereochemistry of **1**. The cyclopropane protons showed clear nOe's with signals at δ 0.80 and 0.95 which were assigned to H_3-29 and H_3-18 (irradiation of H-19β at δ 0.31) and to H-2β and H_3-18 (irradiation of H-19α at δ 0.54). The H_3-18 showed nOe's with both H-19β and H-19α as well as H-20, and H-20 gave nOe with H-22, indicating the β configuration for both the protons at C-20 and C-22. Thus, the structure of **1** was concluded to be (22*R*)-cycloart-25-ene-3β,22-diol [**1**]. This conclusion was fully supported by the ^{13}C-nmr spectrum and by the one-bond ^{1}H-^{13}C heteronuclear chemical shift correlation spectrum (hetero-COSY). Signals of C-3, C-19, C-20, C-21, C-22, C-24, C-25, C-26, and C-27 in the ^{13}C-nmr spectrum were correlated with the chemical shifts of their respective protons in the ^{1}H-nmr spectrum.

Compound **2**, mp 169–170°, $[\alpha]^{25}D$ −18°, showed a molecular ion peak in hrms at *m/z* 442.3866 corresponding to $C_{30}H_{50}O_2$ (calcd 442.3980). The ir

spectrum suggested two hydroxyl groups (3580 and 3440 cm^{-1}), a cyclopropane ring (3045 cm^{-1}), and a terminal methylene group (1640 and 890 cm^{-1}). The mass spectrum was very similar to that of **1**, showing characteristic peaks at *m/z* 355, 302, 315, 297, and 175. The only notable differences were the absence of an $[M-\text{side chain}-2H]^+$ ion, and a McLafferty elimination was also not observed. Compound **2** is, therefore, an isomer of **1**. The ^1H-nmr (500 MHz) spectrum of **2** showed signals due to six tertiary methyl groups (δ 0.86, 0.88, 0.96, 0.98, 1.31, and 1.33) and a pair of doublets at δ 0.30 and 0.50 ($J=4.52$ Hz) for cyclopropane methylene protons. A double doublet at δ 3.20 ($J=9.9$ and 4.5 Hz) was indicative of a proton geminal to a hydroxyl group, and broad singlets at δ 4.60 and 4.70 (1H each) could be assigned to the terminal methylene protons. The BB ^{13}C-nmr and DEPT experiments revealed the presence of six methyl, thirteen methylene, four methine, and seven quaternary carbon atoms.

The carbinol methine proton at δ 3.20 was assigned to C-3 by analogy with compound **1**, and the large magnitude of its coupling constants reflected the β and equatorial configuration of the hydroxyl group at position 3. This conclusion was further supported by the ^{13}C-nmr spectrum of **2**, which showed expected shielding and deshielding effects of the 3β-hydroxyl group on various carbon atoms of ring A [12,13]. The ^1H-nmr spectrum of **2** showed a signal for only one carbinol methine proton, suggesting that the second hydroxyl group was tertiary. This was also confirmed by ^{13}C nmr, which showed one CH at 76.82 and a quaternary carbon at δ 70.97 characteristic for carbons bearing a hydroxyl group. Possible positions of the hydroxyl group were C-20 or C-25. The former possibility was eliminated by the presence of an α-hydroxy isopropyl moiety (δ 1.31 and 1.33 each s, 3H). The terminal methylene group must, therefore, be at C-20. This conclusion was also supported by the mass spectrum in which the peak resulting from loss of the side chain plus two hydrogen atoms was absent. Further evidence was provided by nOe interactions. The H-19β showed nOe interaction with H_3-29 at δ 0.86 and H_3-18 at δ 0.96, and the latter in turn showed nOe interactions with H_a-21 at δ 4.70 and H_2-12 at δ 1.48. The H_b-21 showed nOe interaction with H_2-22 at δ 2.14. Conclusive evidence for the structure of **2** was provided by catalytic hydrogenation of **2** in HOAc over PtO_2 which led to the saturated dihydrodiol of **2**. Physical data of the saturated dihydrodiol were found to be identical with those of 25-hydroxy cycloartanol, which is the corresponding reduction product of cycloart-23-ene-3β,25-diol reported from *Tillandsia usneoides* by Djerassi and McCrindle (14). The structure of **2** is thus cycloart-20-ene-3β,25-diol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded on a JASCO A-302 spectrophotometer. The hrms were recorded on a Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. Nmr spectra were recorded on a Bruker AM-500 spectrometer with TMS as the internal reference. The DEPT experiments were carried out with last pulse angle θ = 45°, 90°, and 135°. The quaternary carbons were determined by subtracting these spectra from the BB ^{13}C-nmr spectrum. The 2D COSY-45° experiment was acquired at 400 MHz with a sweep width of 4000 Hz (2K data points in ω2) and 2000 Hz (256 t_1 values zero-filled to 1K) in ω1. The heteronuclear 2D ^1H-^{13}C chemical shift correlation experiments were carried out at 400 MHz with a sweep width of 12820 Hz (2K data points in ω2) and 1024 Hz (256 t_1 values zero-filled to 1K) in ω_1. In both the 2D experiments a 2-sec relaxation delay was used, and 16 transients were performed for each t_1 value. For nOe measurements, the sample was frozen under liquid N_2 and degassed using a decoupler power of 35 low and pre-irradiation time of 11 sec. An impulse length of 10 msec was maintained to avoid saturation.

PLANT MATERIAL.—*A. ramosa* was collected in Karachi, Pakistan and was identified by Prof.

Dr. S.I. Ali, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen is deposited.

EXTRACTION AND ISOLATION OF TRITERPENOIDS.—The freshly collected whole plant material (20 kg) was extracted with MeOH at room temperature. The MeOH extract was evaporated under reduced pressure, and the residue thus obtained was partitioned between EtOAc and H_2O. The EtOAc fraction (230 g) was subjected to mplc over Si gel (70–230 mesh, E. Merck, 4500 g) and successively eluted with increasing polarities of a mixture of C_6H_{14} and $CHCl_3$. The eluates from C_6H_{14}-$CHCl_3$ (7:3 and 6:4) yielded crystalline residues, which on repeated crystallization from $CHCl_3$/MeOH gave cycloartanol (95 mg) and a binary mixture of triterpenes. The latter were resolved by flash cc over Si gel (230–400 mesh, E. Merck) using C_6H_{14}-$CHCl_3$ (7:3) as solvent system to yield first an additional quantity of cycloartanol (15 mg) and then cycloart-23-ene-3β,25-diol (25 mg).

The eluate from C_6H_{14}-$CHCl_3$ (5.5:4.5 and 5:5) crystallized from $CHCl_3$/MeOH as colorless needles of **1** (65 mg). The mother liquor of **1** was subjected to preparative tlc over Si gel (GF-254, E. Merck) using C_6H_{14}-$CHCl_3$ (5.5:4.5) as solvent system to yield **2**, which crystallized from C_6H_{14}/$CHCl_3$ as colorless needles (30 mg).

(22R)-Cycloart-25-ene-3β,22-diol [**1**].—Mp 180–181°; $[\alpha]^{20}D$ +20.6° (c = 0.87, $CHCl_3$); ir ($CHCl_3$) ν max 3450–3600, 3045, 1650, 1380, 890 cm^{-1}; hrms *m/z* (rel. int.) $[M]^+$ 442.3824 ($C_{30}H_{50}O_2$) (33), $[M-H_2O]^+$ 424.3706 ($C_{30}H_{48}O$) (46), $[M-H_2O-Me]^+$ 409.3484 ($C_{29}H_{45}O$) (38), $[M-2H_2O]^+$ 391.3325 ($C_{29}H_{43}$) (12), $[M-C_4H_8]^+$ 386.3199 ($C_{26}H_{42}O_2$) (1), $[M-H_2O-C_3H_7]^+$ 381.3155 ($C_{27}H_{41}O$) (8), $[M-H_2O-C_5H_9]^+$ 355.3004 ($C_{25}H_{39}O$) (14), $[M-C_8H_{15}O]^+$ 315.2682 ($C_{22}H_{35}O$) (13), $[M^+C_8H_{17}O]^+$ 313.2654 ($C_{22}H_{33}O$) (10), $[M^+-C_9H_{16}O]^+$ 302.2621 ($C_{21}H_{34}O$) (42), $[M-C_8H_{15}O-H_2O]^+$ 297.2562 ($C_{22}H_{33}$) (11), $[M-C_9H_{16}O-H_2O]^+$ 284.2528 ($C_{21}H_{32}$) (15), $[M-C_9H_{16}O-C_8H_{15}O]^+$ 175.1465 ($C_{13}H_{19}$) (58); ^1H nmr ($CDCl_3$) δ 4.80 and 4.49 (br s, H_3-26), 4.06 (ddd, J = 6.6, 6.2, 1.0 Hz, H-22), 3.20 (dd, J = 9.8, 4.4 Hz, H-3), 1.60 (s, H_3-27), 1.43 (dq, J = 6.4, 1.0 Hz, H-20), 0.96 (s, H_3-30), 0.95 (s, H_3-18), 0.88 (s, H_3-28), 0.87 (d, J = 6.4 Hz, H_3-21), 0.80 (s, H_3-29), 0.54 (d, J = 4.2 Hz, H-19α), 0.31 (d, J = 4.2 Hz, H-19β); ^{13}C nmr (75.3 MHz, $CDCl_3$) δ 31.66 (t, C-1), 30.45 (t, C-2), 78.89 (d, C-3), 40.48 (s, C-4), 47.19 (d, C-5), 21.14 (t, C-6), 28.12 (t, C-7), 47.99 (d, C-8), 20.41 (s, C-9), 26.15 (s, C-10), 26.04 (t, C-11), 35.61 (t, C-12), 45.29 (s, C-13), 48.80 (s, C-14), 32.02 (t, C-15), 26.55 (t, C-16), 52.26 (d, C-17), 18.03 (q, C-18), 29.89 (t, C-19), 36.01 (d, C-20), 18.37 (q, C-21), 76.78 (d, C-22), 28.12 (t, C-23), 32.98 (t, C-24), 149.70 (s, C-25), 111.33 (t, C-26), 17.27 (q, C-27), 19.36 (q, C-28), 25.48 (q, C-29), 14.02 (q, C-30).

OXIDATION OF **1**.—Compound **1** (10 mg) was dissolved in Me_2CO (20 ml) and treated with Jones' reagent (2.5 ml) at room temperature. Usual workup provided a diketone (5.6 mg): $[\alpha]^{20}D$ +39.5° ($CHCl_3$, c = 0.054); ir ($CHCl_3$) ν max 3045, 1690–1700, 1640, 1380, 890 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 438 (43), $[M-Me]^+$ 423 (22), $[M-C_4H_8]^+$ 382 (8), $[M-C_8H_{13}O]^+$ 313 (13), $[M-C_8H_{15}O]^+$ 311 (6), $[M-C_9H_{14}O]^+$ 300 (56), $[M-C_9H_{14}O-C_8H_{13}O]^+$ 175 (66).

Cycloart-20-ene-3β,25-diol [**2**].—Mp 169–170°; $[\alpha]^{25}D$ −18° ($CHCl_3$, c = 0.03); ir ($CHCl_3$) ν max 3580, 3440, 3045, 1380, 1640, 890 cm^{-1}; hrms *m/z* (rel. int.) $[M]^+$ 442.3866 ($C_{30}H_{50}O_2$) (30), $[M-H_2O]^+$ 424.3728 ($C_{30}H_{48}O$) (45), $[M-H_2O-Me]^+$ 409.3481 ($C_{29}H_{45}O$) (36), $[M-2H_2O]^+$ 409.3481 ($C_{29}H_{45}O$) (36), $[M-2H_2O]^+$ 406.3666 ($C_{30}H_{46}$) (12), $[M-2H_2O-Me]^+$ 391.3311 ($C_{29}H_{43}$) (10), $[M-H_2O-C_3H_7]^+$ 381.3129 ($C_{27}H_{41}$) (6), $[M-H_2O-C_5H_9]^+$ 355.3062 ($C_{25}H_{39}O$) (1), $[M-C_8H_{15}O]^+$ 315.2701 ($C_{22}H_{35}O$) (17), $[M-C_9H_{16}O]^+$ 302.2650 ($C_{21}H_{34}O$) (40), $[M-C_8H_{15}O-H_2O]^+$ 297.2573 ($C_{22}H_{33}$) (11), $[M-C_9H_{16}O-H_2O]^+$ 284.2524 ($C_{21}H_{32}$) (13), $[M-C_9H_{16}O-C_8H_{15}O]^+$ 175.1483 ($C_{13}H_{19}$) (55); ^1H-nmr ($CDCl_3$) δ 4.70 and 4.60 (br s, H_2-21), 3.20 (dd, J = 9.9, 4.5 Hz, H-3), 2.14 (m, H_2-22), 1.33 and 1.31 (s, H_3-26 and H_3-27), 0.98 (s, H_3-30), 0.96 (s, H_3-18), 0.88 (s, H_3-28), 0.86 (s, H_3-29), 0.50 (d, J = 4.5 Hz, H-19α), 0.30 (d, J = 4.5 Hz, H-19β); ^{13}C nmr (75.3 MHz, $CDCl_3$) δ 31.87 (t, C-1), 30.26 (t, C-2), 76.82 (d, C-3), 40.36 (s, C-4), 47.11 (d, C-5), 21.09 (t, C-6), 28.12 (t, C-7), 47.92 (d, C-8), 20.32 (s, C-9), 26.17 (s, C-10), 26.02 (t, C-11), 35.66 (t, C-12), 45.51 (s, C-13), 48.47 (s, C-14), 33.03 (t, C-15), 26.50 (t, C-16), 51.97 (d, C-17), 18.23 (q, C-18), 29.90 (t, C-19), 156.49 (s, C-20), 106.69 (t, C-21), 33.04 (t, C-22), 34.29 (t, C-23), 39.19 (t, C-24), 70.97 (s, C-25), 29.39 (q, C-26), 29.46 (q, C-27), 19.30 (q, C-28), 25.31 (q, C-29), 14.89 (q, C-30). The assignments were made with the help of hetero-COSY as well as comparison with related triterpenes (15,16).

REDUCTION OF **2**.—Compound **2** (18 mg) in HOAc (1.5 ml) was hydrogenated over PtO_2 (10 mg). The product freed from catalyst and solvent was chromatographed over activated Al_2O_3 (grade I, 10 g). Elution with Et_2O-MeOH (10:1) afforded the saturated dihydrodiol, which crystal-

lized from C_6H_6 as colorless prisms (11 mg): mp 185–186° [lit. (12) 187–188°]; $[\alpha]^{25}D$ +44.6° ($CHCl_3$, $c = 0.07$) [lit. (12) +45° ($CHCl_3$)]; ir ν max 3570, 3450, 3045 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 444 (23), $[M-Me]^+$ 429 (16), $[M-H_2O]^+$ 426 (27), $[M-H_2O-Me]^+$ 411 (19), $[M-2H_2O]^+$ 408 (11), $[M-H_2O-C_5H_9]^+$ 357 (13), $[M-C_8H_{17}O]^+$ 315 (14), $[M-C_9H_{16}O]^+$ 304 (44), $[M-C_8H_{17}O-H_2O]^+$ 297 (12), $[M-C_9H_{16}O-H_2O]^+$ 286 (18), $[M-C_9H_{16}O-C_8H_{17}O]^+$ 175 (69).

Cycloartanol.—Mp 102–103°; $[\alpha]^{20}D$ +49.9° ($CHCl_3$, $c = 0.15$); ms *m/z* (rel. int.) $[M]^+$ 428 (14), $[M-Me]^+$ 413 (19), $[M-H_2O]^+$ 410 (28), $[M-H_2O-Me]^+$ 395 (15), $[M-H_2O-C_5H_9]^+$ 341 (16), $[M-C_8H_{17}]^+$ 315 (25), $[M-C_9H_{16}O]^+$ 288 (30), $[M-C_8H_{17}-H_2O]^+$ 297 (35), $[M-C_9H_{16}O-C_8H_{17}]^+$ 175 (45). The physical and spectral data coincided with those reported in literature (17,18).

Cycloart-23-ene-3β,25-diol.—Mp 198–199°; $[\alpha]^{20}D$ +33.95° ($CHCl_3$, $c = 0.10$); ms *m/z* (rel. int.) $[M]^+$ 442 (12), $[M-Me]^+$ 427 (10), $[M-H_2O]^+$ 424 (17), $[M-H_2O-Me]^+$ 409 (21), $[M-2H_2O-Me]^+$ 391 (19), $[M-H_2O-C_5H_9]^+$ 355 (10), $[M-C_8H_{15}O]^+$ 315 (35), $[M-C_8H_{15}O-2H]^+$ 313 (18), $[M-C_9H_{16}O]^+$ 302 (42), $[M-C_8H_{15}O-H_2O]^+$ 297 (11), $[M-C_9H_{16}O-H_2O]^+$ 284 (18), $[M-C_8H_{15}O-C_9H_{16}O]^+$ 175 (80). The physical and spectral data coincided with those reported in literature (19,20).

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