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Structure of Manevalic and Azizic Acids, Two New Triterpenes from *Cornulaca monacantha* Del.

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Two new triterpene acids were isolated from the plant *Cornulaca monacantha* Del. The first acid was assigned the structure 3β,6α-dihydroxyolean-12-en-27-oic acid I and was given the trivial name manevalic acid. The other compound was assigned the structure 3β,6α-dihydroxyolean-12-en-27,28-dioic acid II and named azizic acid. These assignments were based on spectral and chemical studies. The chemical correlation between manevalic acid and the known triterpene astilbic acid, 3β,6β-dihydroxyolean-12-en-27-oic acid, was established through oxidation of their methyl esters to methyl 3,6-dioxoolean-12-en-27-oate IC.

Keywords——Chenopodiaceae; *Cornulaca monacantha* Del.; triterpenes; manevalic acid, 3β,6α-dihydroxyolean-12-en-27-oic; azizic acid, 3β,6α-dihydroxyolean-12-en-27,28-dioic acid; methyl 3,6-dioxoolean-12-en-27-oate

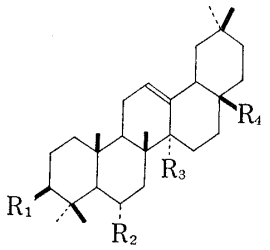
We reported earlier on the isolation and partial characterization of three new triterpene acids[2] from *Cornulaca monacantha* Del., an Egyptian desert plant belonging to the family Chenopodiaceae. Reinvestigation of the same plant on a large scale resulted in the isolation of two additional new triterpene acids after acid hydrolysis of the natural glycosides. This report deals with the isolation and structure elucidation of these two new compounds from the title plant.

The dry powdered plant material (leaves and small branches) was defatted, followed by repeated extraction with refluxing ethanol. The glycoside fraction of the extractives was hydrolyzed with hydrochloric acid in ethanol. Chromatography of the aglycone mixture thus obtained on neutral alumina did not effect resolution, but removed most of the impurities and pigments. The pure compounds were isolated and purified by preparative layer chromatography.

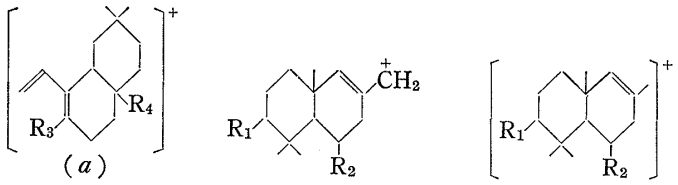
Compound I (manevalic acid) was obtained as fine needles, mp 269—272° and was assigned the formula $C_{30}H_{48}O_4$ based on elemental analysis and mass spectrometric data (M^+, *m/e* 472). The infrared (IR) spectrum of the compound showed absorption bands at 3400, 3300 (hydroxyl groups) and 1690 cm^{-1} (carbonyl). The compound formed a methyl ester derivative IA. The mass spectrum of IA showed a base peak at *m/e* 262 (base peak in I at *m/e* 248). Confirmation of the presence of the carboxyl group in manevalic acid was provided by the presence of a three-proton singlet at δ 3.60 ppm in the nuclear magnetic resonance (NMR) spectrum of compound IA, indicating a carbomethoxyl group. The presence of two acylable hydroxyl groups in compound I was proved by acetylating compound IA with acetic anhydride/pyridine, which gave an acetate IB. A molecular ion peak, M^+, at *m/e* 570 indicated two acetyl groups and this was confirmed by the NMR of the acetate which exhibited two signals at δ 2.00 (3H, s) and 2.03 (3H, s) ppm. The mass spectrum of manevalic acid I showed a base peak at *m/e* 248 (ion a) resulting from retro–Diels–Alder

1) Location: *a) Mansoura, Dakahliah, Arab Republic of Egypt; b) Hittorfstr. 58-62, 4400-Münster/Westf.* (BRD); *c) Dokki, Cairo.*

2) M.A. Amer, A.M. Dawidar, and M.B.E. Fayez, *Planta Medica*, **26**, 289 (1974).



I: $R_1=R_2=OH$; $R_3=COOH$; $R_4=CH_3$
IA: $R_1=R_2=OH$; $R_3=COOMe$; $R_4=CH_3$
IB: $R_1=R_2=OAc$; $R_3=COOMe$; $R_4=CH_3$
IC: $R_1=R_2=O$; $R_3=COOMe$; $R_4=CH_3$
II: $R_1=R_2=OH$; $R_3=R_4=COOH$
IIA: $R_1=R_2=OH$; $R_3=R_4=COOMe$
IIB: $R_1=R_2=OAc$; $R_3=R_4=COOMe$
IIC: $R_1=R_2=O$; $R_3=R_4=COOMe$



(a) (b) (c)

Chart 1

fragmentation of ring C, favoring the presence of a double bond at C-12 and the attachment of the carboxyl group to ring C, D or E of the molecule.[3] The presence of ions b and c at *m/e* 223 and 224, respectively, suggested the location of the two hydroxyl groups at rings A and/or B.[3] Assuming one of the hydroxyl groups to be located at C-3, the second was proved not to be involved in a glycol system by the failure of periodate cleavage and acetonide formation attempts,[4] and tertiary and axially bound positions were excluded by the rate of esterification.[5] The second hydroxyl group could thus be 6α or 7β attached to ring B. Position 1 is most unlikely, because steroids and triterpenoids hydroxylated at C-1 are very rare in nature. The carboxyl group in manevalic acid was probably not attached to C-20, due to the high intensity (*ca.* 80%) of the ion at *m/e* 203 resulting from expulsion of the carbomethoxyl substituent from ion a in the mass spectra of compounds IA, IB. This assumption was based on the findings of Budzkiwicz *et al.*[3] It was thought at this stage that manevalic acid might be 3β,6α (or 7β)-dihydroxy-18ξ-olean-12-ene-27 (or 28)-oic acid.

Takahashi *et al.*[6] reported the isolation of astilbic acid from the roots of *Astilbe thunbergii* and proved its structure to be 3β,6β-dihydroxyolean-12-ene-27-oic acid. The structural relationship between astilbic acid and manevalic acid was determined through the identity of the oxidation product IC obtained from methyl manevalate with an authentic sample[7] of methyl 3,6-dioxoolean-12-en-27-oate (mixed mp and IR). Consequently manevalic acid was established to be 3β,6α-dihydroxyolean-12-en-27-oic acid and formulated as I, *cf.* Chart 1.

The ^{13}C NMR chemical shifts (δ_c) of methyl manevalate were measured, assigned tentatively and are listed in Table I. They were valuable in locating the various functional groups in the oleanane carbon skeleton.

The other isolated compound, azizic acid II, was obtained as fine needles, mp 259—263°, and was assigned the formula $C_{30}H_{46}O_6$ based on analytical and spectral data. Compound II formed dimethyl IIA and diacetate IIB esters, indicating the presence of two carboxyl and two hydroxyl groups in the molecule. Azizic acid was structurally related to manevalic acid on the basis of inspection and comparison of the spectral data for compounds IA, IB, IIA and IIB. The base ion at *m/e* 262 found in the mass spectra of IA and IB was shifted to *m/e* 306 in the case of azizic acid derivatives IIA and IIB. The presence of the

3) H. Budzikiewicz, J.M. Wilson, and C.D. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
4) M.H.A. Elgamal and M.B.E. Fayez, *Z. Anal. Chem.*, **226**, 408 (1967).
5) M.H.A. Elgamal and M.B.E. Fayez, *Acta Chim.* (Budapest), **58**, 75 (1968).
6) K. Takahashi, K. Kanayama, Y. Tanabe, and M. Tori, *Chem. Pharm. Bull.* (Tokyo), **20**, 2106 (1972).
7) Provided by Prof. K. Takahashi, Kanazawa University, Japan, to whom we are indebted.

Table I. ^{13}C Chemical Shifts[a] (in $CDCl_3$, $\delta_C^{TMS}=0$) of Methyl Manevalate IA and Methyl Azizate IIA

	Manevalate	Azizate	Oleanolate[b]	Ursolate[b]
C- 1	38.2	38.1	38.5	38.8
C- 2	26.7	26.7	27.1	27.3
C- 3	76.8	76.6	78.7	78.8
C- 4	41.8	43.7	38.7	38.8
C- 5	49.8	49.7	55.5	55.4
C- 6	71.9	71.8	18.3	18.4
C- 7	32.5	32.5	32.6	33.0
C- 8	39.4	39.3	39.3	39.0
C- 9	47.6	47.6	47.6	47.5
C-10	36.9	36.9	37.0	37.0
C-11	25.9	25.9	23.1	16.9
C-12	122.3	123.0	122.1	125.5
C-13	143.8	143.1	143.4	138.0
C-14	51.4	51.7	41.6	42.0
C-15	29.6	29.6	27.7	28.2
C-16	23.4	28.3	23.4	24.3
C-17	26.7	51.4	46.6	48.1
C-18	41.7	42.5	41.3	52.8
C-19	45.9	46.1	45.8	39.1
C-20	30.6	30.4	30.6	38.8
C-21	33.9	42.1	33.8	30.7
C-22	32.4	41.5	32.3	36.7
C-23	27.7	27.7	28.1	28.2
C-24	16.9	16.8	15.6	15.5
C-25	15.6	15.6	15.3	15.7
C-26	18.5	18.4	16.8	16.9
C-27	178.3	177.1	26.0	23.3
C-28	15.6	177.6	177.9	177.7
C-29	33.0	33.5	33.1	23.6
C-30	23.6	23.3	23.6	21.2

a) Recorded on a Brucker WH-90 pulse FT-spectrometer at 22.63 MHz in 10 mm o.d. spinning tubes; conc., 0.1—0.2 mmol/cm; spectral width, 6000 Hz; pulse width, 2.8 sec.
b) Data taken from ref. 8).

two carboxyl groups was confirmed by the NMR spectrum of the methyl ester diacetate IIB which revealed the presence of two carbomethoxyl groups (δ 3.57, 3H, s; δ 3.63, 3H, s). The spectrum also showed two acetyl groups at δ 2.00 (3H, s) and δ 2.03 (3H, s). The two hydroxyl groups in azizic acid were proved to be secondary, since oxidation of methyl azizate IIA gave a diketo product IIC, $C_{32}H_{46}O_6$ (M^+ at m/e 526), mp 194—197°, which exhibited no hydroxylic function in the IR region near 3400 cm^{-1}. They were shown not to be involved in a glycolic system and proved to be equatorial, 3β,6α or 3β, 7β-, as in the case of manevalic acid. ^{13}C NMR of methyl azizate IIA clearly suggested the presence of 3β-OH (δ_c 76.6 ppm) and 6α-OH (δ_c 71.8 ppm) in azizic acid, in accord with those of methyl manevalate, *cf.* Table I. The double bond was shown to be between C-12 (δ_c 123.0) and C-13 (δ_c 143.7) in an olean-12-ene carbocyclic skeleton.[8] In order to assist the location of the two carbomethoxyl groups in product IIA, the ^{13}C resonance of methyl glycyrrhetate (methyl 3β-hydroxy-11-oxoolean-12-en-30-oate), a model compound which has a carbomethoxyl group attached to C-20, was measured. The spectrum showed a signal at δ_c 169.0 ppm due to C-30 $COOCH_3$. This led us to exclude the C-20 position in methyl azizate IIA and to assign the two carbomethoxyl groups, δ_c 177.1 to C-14 and δ_c 177.6 to C-17. Based on these results, it was proposed that azizic acid is 3β,6α-dihydroxyolean-12-ene-27,28-dioic acid, formulated as II.

8) S. Soe, Y. Tomita, and K. Tori, *Tetrahedron Lett.*, **1975**, 7.

Experimental[9]

Thin-Layer Chromatography——Silica gel G (Merck), 0.25 mm thick, was used as a stationary phase for both analytical and preparative thin-layer chromatography (TLC). The solvent system was: light petroleum–benzene–ethyl acetate–acetic acid (10: 20: 6: 0.5). The spots were located using iodine vapor. The chromatograms were visualized by spraying with chlorosulfonic acid in AcOH (1: 3 v/v).

Isolation of the Triterpene Acids——The dry powdered plant material (leaves and small branches of *C. monacantha* DEL., (3 kg)) was defatted by repeated extraction with hot light petroleum (bp 60—80°). The residue was subsequently extracted with hot ethanol (80%, total Vol. 15 l). The extract was concentrated (to 4 l) then treated with conc. HCl (to provide a 4 N soln). The reaction mixture was refluxed for one hr. Water (4 l) was added after cooling; a brown amorphous powder deposited, and was filtered off, then dried (50 g). TLC showed five spots having *Rf* 0.51, 0.42, 0.35, 0.16 (manevalic) and 0.11 (azizic). The aglycone mixture was chromatographed on a neutral alumina column (800 g, 60×5 cm) by homogenizing it with a portion of alumina (100 g) then poured onto the top of the column and eluted with a series of $CHCl_3$ and $CHCl_3$ containing 0.5%, 1%, 1.5% and 2% MeOH. The resolution was unsuccessful, but the sapogenin mixture was purified from many pigments and colored matter. Fractionation of the triterpenoids was effected by TLC in the usual manner. The compounds were eluted from the silica gel with 5% EtOH in $CHCl_3$. The fractionated terpene acids showed red, violet then blue colors in the Liebermann-Burchard test and showed a yellow color with tetranitromethane in $CHCl_3$.

Manevalic Acid I——It was crystallized from aq. MeOH as fine needles, mp 269—272°, $[\alpha]_D$ +58° (c=0.42 in pyridine). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 3300 (broad shoulder, OH), 1690 (COOH), 1385, 1360 (A-region), 1315, 1300 and 1265 (B-region).[10] Mass Spectrum (MS) *m/e* (%): 472 (9) (M^+, $C_{30}H_{48}O_4$), 457 (6) (M^+-CH_3), 454 (16) (M^+-H_2O), 436 (14) (M^+-2H_2O), 426 (39) ($M^+-HCOOH$), 248 (100) (*a*), 223 (8) (*b*), 224 (6) (*c*) and 203 (*a*-COOH). *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.27%; H, 10.17%. Found: C, 76.40%; H, 10.30%.

Treatment of manevalic acid with potassium periodate,[4] conducted on the chromatoplates, revealed that the compound was unaffected under conditions which cause cleavage of model triterpenoid glycols (*e.g.* commic acid and hederagenin). The compound was also indifferent to the action of acetone in the presence of 10% H_2SO_4 performed on the chromatoplates[4] under conditions which gave acetonide derivatives with model triterpenoid glycols.

Methyl Manevalate IA——Manevalic acid (50 mg) was dissolved in MeOH (10 ml) and $CHCl_3$ (10 ml) and treated with an ethereal solution of CH_2N_2 (10 ml) at 0° for about 2 hr. The reaction mixture was evaporated to dryness, then crystallized twice from aq. MeOH to give clusters of fine needles of methyl manevalate IA (35 mg), mp 218—221°, $[\alpha]_D$ +57° ($CHCl_3$, c=0.45). IR ν_{max}^{KBr} cm^{-1}: 3400 (broad, OH), 1730 and 1260 (ester). MS *m/e* (%): 486 (14) (M^+, $C_{31}H_{50}O_4$), 471 (5) (M^+-CH_3), 468 (10) (M^+-H_2O), 456 (9) (M^+-2CH_3), 426 (9) ($M^+-HCOOCH_3$), 262 (100) (*a*), 223 (19) (*b*), 224 (5) (*c*) and 203 (83) (*a*-$COOCH_3$). NMR: δ 0.66 (3H, s), 0.87 (12H, s), 0.89 (3H, s), 1.09 (3H, s), 3.60 (3H, s, $COOCH_3$) and 5.24 (1H, t, olefinic, J= 4 Hz).

Acetylation of IA on chromatoplates[5] showed partial conversion to a monoacetate and diacetate within 30 minutes, with a reduced amount of the former after 60 minutes and only the latter after 90 minutes.

Methyl Manevalate Diacetate IB——Product IA (50 mg) was dissolved in pyridine (5 ml) and Ac_2O (5 ml). The reaction mixture was left on a water-bath for 120 minutes. Aq. MeOH (50%) was added dropwise, and shiny plates deposited; these were filtered and crystallized from aq. MeOH to give the diacetate IB as lustrous plates (40 mg), mp 188—191°, $[\alpha]_D$ +68° ($CHCl_3$, c=0.47). IR ν_{max}^{KBr} cm^{-1}: 1730 and 1240 (ester). MS *m/e* (%): 570 (13) (M^+-2CH_3COOH), 262 (100) (*a*), 307 (10) (*b*), 308 (*c*) and 203 (81) (*a*-$COOCH_3$). NMR: δ 0.73 (3H, s), 0.82 (3H, s), 1.01 (6H, s), 1.04 (6H, s), 1.19 (3H, s), 2.00 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO) and 3.60 (3H, s, OCH_3). *Anal.* Calcd. for $C_{35}H_{54}O_6$: C, 73.68%; H, 9.74%. Found: C, 73.14%; H, 9.49%.

Oxidation of Methyl Manevalate——A solution of IA (20 mg) in pyridine (1 ml) was treated with CrO_3-pyridine mixture (250 mg CrO_3 in pyridine 6 ml), and the solution was agitated for 120 minutes at 0°, then kept at room temperature for 24 hours. The mixture was poured into ice-H_2O, extracted with ether and processed in the usual manner to give a brownish residue (15 mg), which was purified on a silica column (20 gm), eluted with benzene, and crystallized from $CHCl_3$/MeOH to give needles of product IC, mp and mixed mp with methyl 3,6-dioxoolean-12-en-27-oate, 179—183°, identical IR spectra. IR ν_{max}^{KBr} cm^{-1}: 1720 ($COOCH_3$, shoulder) and 1700 (>C=O).

Azizic Acid II——It was obtained as needles from aq. MeOH, mp 259—263°, $[\alpha]_D$ +98° (pyridine, c= 0.67). IR ν_{max}^{KBr} cm^{-1}: 3400 (broad, OH), 1720, 1690 (shoulder, COOH), 1385, 1360 (A-region), 1315, 1295 and

9) Melting points were determined using a Kofler hot-stage instrument and are uncorrected. IR spectra were recorded using a Perkin-Elmer 457 spectrophotometer. NMR spectra were recorded at 60 MHz with a Varian T-60A instrument, in $CDCl_3$, using tetramethylsilane as an internal standard. Mass spectral data were obtained with a Hitachi Perkin-Elmer RMU-6D spectrophotometer. Optical rotations were measured using a Perkin-Elmer 241 polarimeter.

10) G. Snatzke, F. Lampert, and R. Tschesche, *Tetrahedron*, **18**, 1417 (1962).

1270 (B-region). *Anal.* Calcd. for $C_{30}H_{46}O_6$: C, 71.71%; H, 9.16%. Found: C, 72.30%; H, 9.19%.

Methyl Azizate IIA——Azizic acid (50 mg) was dissolved in MeOH (10 ml) and $CHCl_3$ (10 ml) and treated with an ethereal solution of CH_2N_2 (10 ml) at 0° for about 2 hr. The reaction mixture was evaporated to dryness, then crystallized from aq. MeOH to give colorless fine needles (40 mg) of methyl azizate, mp 209—212°, $[\alpha]_D$ +86° ($CHCl_3$, c=0.52). IR ν_{max}^{KBr} cm^{-1}: 3450, 3350 (OH), 1730, 1715 (shoulder) and 1260 (ester). MS *m/e* (%): 530 (10) (M^+, $C_{32}H_{50}O_6$), 515 (5) (M^+-CH_3), 512 (9) (M^+-H_2O), 494 (16) (M^+-2H_2O), 471 (20) ($M^+-COOCH_3$), 470 (40) ($M^+-HCOOCH_3$), 306 (100) (*a*), 247 (79) (*a*-$COOCH_3$), 223 (20) (*b*), 224 (8) (*c*) and 188 (81) (*a*-2$COOCH_3$). NMR: δ 0.69 (3H, s), 0.84 (3H, s), 0.9 (3H, s), 1.08 (6H, s), 1.17 (3H, s), 3.54 (3H, s, $COOCH_3$), 3.63 (3H, s, $COOCH_3$) and 5.26 (1H, t, J=4 Hz, olefinic). *Anal.* Calcd. for $C_{32}H_{50}O_6$: C, 72.45%; H, 9.43%. Found: C, 72.85%; H, 9.72.

Methyl Azizate Diacetate IIB——Compound IIA (50 mg) was dissolved in pyridine (5 ml) and Ac_2O (5 ml). The reaction mixture was left on a water-bath for 120 minutes. Aq. MeOH (50%) was added dropwise, and shiny plates deposited; these were filtered and crystallized from aq. MeOH to give the diacetate (42 mg) as lustrous plates, mp 252—255°, $[\alpha]_D$ +81° ($CHCl_3$, c=0.65). IR ν_{max}^{KBr} cm^{-1}: 1730 and 1240 (ester). MS *m/e* (%): 614 (10) (M^+, $C_{36}H_{54}O_8$), 599 (6) (M^+-CH_3), 494 (16) (M^+-2CH_3COOH), 306 (100) (*a*), 307 (40) (*b*), 308 (9) (*c*), 247 (78) (*a*-$COOCH_3$) and 188 (80) (*a*-2$COOCH_3$). NMR: δ 0.74 (3H, s), 0.86 (3H, s), 0.98 (3H, s), 1.13 (12H, s), 2.00 (3H, s, $C\underline{H}_3COO$), 2.03 (3H, s, $C\underline{H}_3COO$), 3.57 (3H, s, $COOC\underline{H}_3$), 3.63 (3H, s, $COO\underline{CH}_3$), 4.74 (1H, t, J=6 Hz, C-3H) and 5.34 (1H, t, J=4 Hz, olefinic). *Anal.* Calcd. for $C_{36}H_{54}O_8$: C, 70.35%; H, 8.79. Found: C, 70.54%; H, 8.88%.

Oxidation of Methyl Azizate——A solution of IIA (20 mg) in pyridine (1 ml) was treated with CrO_3–pyridine mixture (250 mg of CrO_3 in 6 ml of pyridine and the mixture was agitated for 120 minutes at 0°, then kept at room temperature for 24 hr. The reaction mixture was processed in exactly the same manner as with the oxidation of methyl manevalate to give the diketonic product IIC as colorless fine needles (8 mg), mp 194—197°. IR ν_{max}^{KBr} cm^{-1}: 1725, 1160 (ester) and 1710 (carbonyl). MS *m/e* (%): 526 (8) (M^+, $C_{32}H_{46}O_6$), 511 (7) (M^+-CH_3), 467 (70) ($M^+-COOCH_3$) and 408 (80) ($M^+-2COOCH_3$).

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