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SESQUITERPENE ACIDS FROM *DITTRICHIA VISCOSA*

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Key Word Index—*Dittrichia viscosa*; Compositae; Inuleae; sesquiterpenes.

Abstract—*Dittrichia viscosa* afforded in addition to compounds reported previously, ilicic acid and two new sesquiterpene acids. The structures of the new compounds were established by chemical and spectral methods.

INTRODUCTION

Previous work on the sesquiterpenoids constituents of *Dittrichia viscosa* (L.) W. Greuter subsp. *viscosa* [sin. *Inula viscosa* (L.) Aiton] led to the identification of 12-carboxyeudesma-3,11(13)-diene (**1a**) [1], 2-deacetoxyxanthinin, inuvisculide [2], and germacranolides [3]. The present paper describes the isolation, from the same plant material, of ilicic acid (**2**) [4–10] and of two new sesquiterpene acids **3a** and **4a**.

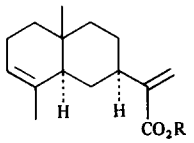
The structures of the new sesquiterpenoids, 3α-hydroxycostic acid (**3a**) and 2α-hydroxy-3,4-dehydro-4,15-dihydrocostic acid (**4a**) were determined by spectroscopic data and chemical correlation with closely related compounds.

RESULTS AND DISCUSSION

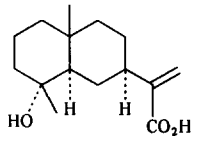
Chromatographic separation of the acid components of the extract of the plant led to the isolation, in the less polar fraction, of a mixture of compounds **2**, **3a** and **4a**, which gave a single spot on TLC. From a chloroform solution of this fraction ilicic acid (**2**) crystallized on standing for several days at room temperature and was identified by comparison (TLC, mp, IR and ^{1}H NMR) with an authentic sample isolated from *Inula graveolens* [10] Repetitive column chromatography of the material from the mother liquor gave two new acids (**3a** and **4a**), which were purified as their methyl esters (**3b** and **4b**).

Compound **3a**, molecular formula $C_{15}H_{22}O_3$ ($[M]^+$ at m/z 250), colourless gum, showed IR bands assignable to a hydroxyl (3600 cm^{-1}) and carboxylic acid (1690 cm^{-1}). Its ^{1}H NMR spectrum exhibited two narrowly split doublets at δ5.62 and 6.25, a broad singlet centred at 4.29 (on acetylation to yield **3c** this signal shifted downfield to 5.29), two broad singlets at 4.54 and 4.91, and a sharp singlet (3H) at 0.72.

Methylation of compound **3a** with diazomethane gave a methyl ester (**3b**) whose IR spectrum showed a carbonyl band at 1715 cm^{-1}. Pyridinium chlorochromate oxydation of **3b** afforded a conjugated ketone (**3d**), λ_{max} 208 nm (ε38 500) whose IR spectrum exhibited a new carbonyl band at 1690 cm^{-1}. As would be expected, the signals of the unconjugated methylene group of **3a** were now shifted downfield to δ5.07 and 5.85. The hydroxyl group at **3a** must therefore be allylic. Interestingly, the oxidation of **3b** led also to the aldehyde **5** as a minor reaction product. The above data are accommodated most readily by a bicarbocyclic sesquiterpene structure, and in view of the previously described compounds from



1a R = H
1b R = Me



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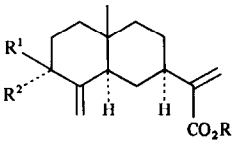
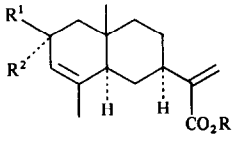
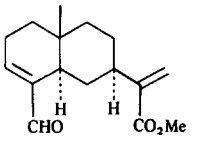


3a R = R^1 = H, R^2 = OH
3b R = Me, R^1 = H, R^2 = OH
3c R = R^1 = H, R^2 = OAc
3d R = Me, R^1, R^2 = O



4a R = R^1 = H, R^2 = OH
4b R = Me, R^1 = H, R^2 = OH
4c R = Me, R^1 R^2 = O
4d R = R^2 = H, R^2 = OAc



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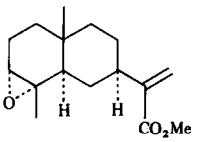

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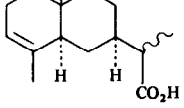

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Inula viscosa, e.g. 12-carboxyeudesma-3,11(13)-diene (**1a**) and ilicic acid (**2**) lead to the tentative structure **3a**. Proof of this structure was obtained by the conversion of compound **1a** to the hydroxy ester **3b** on treatment with *p*-nitroperbenzoic acid. The reaction produced only a small quantity of the expected α-epoxide **6**. The major product, derived from acid-catalysed epoxide ring opening, was **3b**, which was identical in all respects with the methylation product of **3a**, isolated from the natural source. The second new sesquiterpene acid **4a**, molecular formula $C_{15}H_{22}O_3$, ($[M]^+$ at *m/z* 250), was a colourless gum with an IR spectrum showing the presence of a hydroxyl (3600 cm^{-1}) and carboxylic acid (1690 cm^{-1}).

The ^1H NMR spectrum was similar to that of 3α-hydroxycostic acid (**3a**) except for the following differences: (a) the signals for the exocyclic unconjugated methylene protons near δ5 were missing, and instead, a three-proton singlet at δ1.63 was found; (b) the oxymethyne proton appear as a broad multiplet at δ4.27 ($W_{1/2}$ = 19 Hz), indicating that it was in an axial orientation; (c) there was an olefinic signal at δ5.32. Methylation of compound **4a** with diazomethane afforded the ester **4b**, whose pyridinium chlorochromate oxidation gave a conjugated ketone (**4c**), λ_{max} 236 nm (ε 10 200). The IR spectrum of **4c** exhibited a new carbonyl band at 1655 cm^{-1} and the ^1H NMR spectrum showed the olefinic proton shifted downfield to δ5.85. Acetylation of the hydroxy-acid **4a** provided the monoacetate **4d**.

From the above data the new acid has the structure of 2α-hydroxy-3,4-dehydro-4,15-dihydrocostic acid (**4a**). Conclusive evidence in support of this structure came from the reduction of acetate **4d** with lithium in liquid ammonia to an olefinic acid (**8**), identical in all respects with a compound prepared by reduction of the natural acid **1a**.

EXPERIMENTAL

Plant materials were collected in September 1983, near Perugia, Umbria, Italy, and voucher specimens were deposited in the Herbarium of the 'Dipartimento di Biologia Vegetale' of the University of Perugia, Italy. Compounds **1a**, **2**, **3a** and **4a** are present only in plant material collected during the summer.

Extraction and isolation of the components. Dried and finely powdered *Dittrichia* aereal parts (1000 g) were extracted with Me_2CO. The resulting extracts were evaporated at low temp. The crude gum (90 g) was dissolved in $CHCl_3$ and extracted with 1 N NaOH. The oily, coloured acid fraction (22 g) was chromatographed on silica gel (Merck 70–230 mesh ASTM) and elution with $CHCl_3$–MeOH (49:1) afforded 3.1 g of 12-carboxyeudesma-3,11(13)-diene (**1a**), 0.75 g of 2-deacetoxyanthinin [2], and 70 mg of inuvisculide [2]. Subsequent elution with $CHCl_3$–MeOH (24:1) yielded a mixture 4.2 g of **2**, **3a** and **4a**. The mixture on standing in $CHCl_3$ deposited crystals, mp 173–175° (2 g) which were identified as ilicic acid (**2**). The material from the mother liquor was submitted to repetitive column chromatography (*vide supra*) and gave **2** (1.1 g) and a 60:40 mixture (by ^1H NMR criteria) of **3a** and **4a** (0.9 g). The mixtuere of **3a** and **4a** was esterified by addition of CH_2N_2. The reaction products were separated by medium pressure chromatography [pre-packed column size C(440-37) LiChropep Si 60 (40–63 μm)]. Elution with $CHCl_3$–MeOH (99:1) gave **3b** (350 mg) and **4b** (250 mg).

3α-Hydroxycostic acid (**3a**). Isolated as its methyl ester **3b**, colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1720 (CO_2Me); MS *m/z*: 264 $[M]^+$. (Found: C, 72.41; H, 9.23. $C_{16}H_{24}O_3$ requires: C, 72.69; H, 9.15%); ^1H NMR (90 MHz, $CDCl_3$): δ0.74 (3H, *s*, H-14), 3.78 (3H, *s*, CO_2Me), 4.31 (1H, *dd*, $J_{3\beta,2\beta}$ = 3 Hz, $J_{3\beta,2\alpha}$ = 1 Hz, H-3) [11], 4.59, 4.94 (2H, each *br s*, H-15), 5.58, 6.18 (2H, each *br s*, H-13). Compound **3b** (0.2 g) in 30 ml MeOH–H_2O (9:1) containing 1.5 g KOH was refluxed for 2 hr. The usual work-up afforded 160 mg of **3a** as a colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1690 (COOH); MS *m/z*: 250 $[M]^+$. (Found: C, 71.70; H, 8.98. $C_{15}H_{22}O_3$ requires: C, 71.97; H, 8.86%.) ^1H NMR (90 MHz, $CDCl_3$): δ0.72 (3H, *s*, H-14), 4.29 (1H, *dd*, $J_{3\beta,2\beta}$ = 3 Hz, $J_{3\beta,2\alpha}$ = 1 Hz, H-3), 4.54, 4.91 (2H, each *br s*, H-15), 5.62, 6.25 (2H, each *br s*, H-13).

3α-Acetoxycostic acid (**3c**). Acetylation of **3a** (50 mg) in pyridine–Ac_2O for 15 hr, followed by the usual work-up, gave the acetate **3c**, colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (OAc), 1690 (COOH). (Found: C, 69.54; H, 8.38. $C_{17}H_{24}O_4$ requires: C, 69.83; H, 8.27%.) ^1H NMR (90 MHz, $CDCl_3$): δ0.75 (3H, *s*, H-14), 2.05 (3H, *s*, OAc), 4.63, 5.02 (2H, each *br s*, H-15), 5.29 (1H, *dd*, $J_{3\beta,2\beta}$ = 3 Hz, $J_{3\beta,2\alpha}$ = 1 Hz, H-3), 5.52, 6.28 (2H, each *s*, H-13).

Oxidation of methyl-3α-hydroxycostate (**3b**). The ester **3b** (100 mg) was treated in CH_2Cl_2 with pyridinium chlorochromate (100 mg) at room temp. for 1 hr, MeOH was then added, the mixture was poured into H_2O and the products recovered in $CHCl_3$ were chromatographed on silica gel. Elution with C_6H_6 gave a fraction (60 mg), colourless oil, which was a mixture (60:40) of methyl-3-oxocostate (**3d**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680 (CO), 1710 (CO_2Me); ^1H NMR (90 MHz, $CDCl_3$): δ0.98 (3H, *s*, H-14), 3.92 (3H, *s*, CO_2Me), 5.06, 5.86 (2H, each *m*, H-15), 5.56, 6.14 (2H, each *s*, H-13); and aldehyde **5**, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2855, 2720, 1680 (CHO), 1710 (CO_2Me): ^1H NMR: δ1.04 (3H, *s*, H-14), 3.92 (3H, *s*, CO_2Me), 5.62, 6.21 (2H, each *s*, H-13), 6.68 (1H, *m*, H-3), 9.43 (1H, *s*, CHO). (Found: C, 73.41; H, 8.31. $C_{16}H_{22}O_3$ requires: C, 73.25; H, 8.45%.)

Epoxidation of ester **1b**. To a soln of **1b** (1 g) in $CHCl_3$ (100 ml) was added *p*-nitroperbenzoic acid (800 mg) and the reaction mixture was stirred at room temp. for 20 min. The reaction mixture was washed with 1% $NaHSO_3$, satd soln of $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated. Chromatography of the residue on silica gel and elution with $CHCl_3$–MeOH (99:1) gave **6** (150 mg) as a colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (CO_2Me). (Found: C, 72.81; H, 9.01. $C_{16}H_{24}O_3$: requires C, 72.69; H, 9.15%.) ^1H NMR (90 MHz, $CDCl_3$): δ0.92 (3H, *s*, H-14), 1.22 (3H, *s*, H-15), 2.91 (1H, *m*, 3-H), 3.77 (3H, *s*, CO_2Me), 5.53, 6.11 (2H, each *s*, H-13). Further elution gave **3b** (450 mg) identical in all respects with **3b** prepared from acid **3a**.

2α-Hydroxy-3,4-dehydro-4,15-dihydrocostic acid (**4a**). Isolated as its methyl ester **4b**, colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1720 (CO_2Me); MS *m/z*: 264 $[M]^+$. (Found: C, 72.51; H, 9.21. $C_{16}H_{24}O_3$: requires C, 72.69; H, 9.15%.) ^1H NMR (90 MHz, $CDCl_3$): δ0.89 (3H, *s*, H-14), 1.68 (3H, *s*, H-15), 3.81 (3H, *s*, CO_2Me), 4.32 (1H, *m*, H-2), 5.43 (1H, *m*, H-3), 5.58, 6.17 (2H, each *s*, H-13). Compound **4b** (0.2 g) in 30 ml MeOH–H_2O (9:1) containing 1.5 g of KOH was refluxed for 2 hr. Usual work-up afforded 150 mg of **4a** as a colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1690 (COOH); MS *m/z*: 250 $[M]^+$. (Found: C, 71.82; H, 8.95. $C_{15}H_{22}O_3$ requires: C, 71.97; H, 8.86%.) ^1H NMR (90 MHz, $CDCl_3$): δ0.84 (3H, *s*, H-14), 1.62 (3H, *s*, H-15), 4.27 (1H, *m*, H-2), 5.32 (1H, *m*, H-3), 5.56, 6.21 (2H, each *s*, H-13). Further elution with C_6H_6 afforded **3b** (20 mg).

2α-Acetoxy-3,4-dehydro-4,15-dihydrocostic acid (**4d**). Acetylation of **4a** (50 mg) in pyridine–Ac_2O for 15 hr, followed by the usual work-up, gave the acetate **4d**, colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1728 (OAc), 1690 (COOH). (Found: C, 69.93; H, 8.41. $C_{17}H_{24}O_4$ requires: C, 69.83; H, 8.27%.) ^1H NMR (90 MHz, $CDCl_3$): δ0.91 (3H, *s*, H-14), 1.68 (3H, *s*, H-15), 2.05 (3H, *s*, $OCOCH_3$), 5.32 (1H, *m*, H-3), 5.44 (1H, *m*, H-2), 5.65, 6.29 (2H, each *s*, H-13).

Oxidation of **4b**. The ester **4b** (50 mg) was treated in CH_2Cl_2 with pyridinium chlorochromate (50 mg) at room temp. for 1 hr.

MeOH was then added, the mixture was poured into H_2O and the products recovered in $CHCl_3$ were chromatographed on silica gel. Elution with C_6H_6 gave **4c** (38 mg), colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1655 (CO), 1710 (CO_2Me): UV λ_{max}^{MeOH} nm: 236 (ε10 000); ^1H NMR (90 MHz, $CDCl_3$): δ0.94 (3H, *s*, H-13), 1.92 (3H, *d*, J = 2 Hz, H-15), 3.82 (3H, *s*, CO_2Me), 5.62, 6.22 (2H, each *s*, H-13), 5.85 (1H, *m*, H-3). (Found: C, 73.38; H, 8.34. $C_{16}H_{22}O_3$: requires C, 73.25; H, 8.45%.)

Lithium–ammonia reduction of 2α-*acetoxy*-3,4-*dehydro*-4,15-*dihydrocostic acid* (**4d**). A soln of **4d** (100 mg) in THF (3 ml) was added to a stirring soln of Li (50 mg) in liquid NH_3 (10 ml) umder N_2 at −40°, and the mixture was then stirred for 30 min. Enough NH_4Cl was added to discharge the blue colour and the ammonia allowed to evaporate. The residue was shaken with a mixture of H_2O and $CHCl_3$ and the organic layer dried (Na_2SO_4) and evaporated. Chromatography of the residue on silica gel and elution with $CHCl_3$–MeOH (24:1) gave the acid **7** (60 mg), colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (COOH). (Found: C, 76.41; H, 10.11. $C_{15}H_{24}O_2$: requires C, 76.22; H, 10.24%.) ^1H NMR (90 MHz, $CDCl_3$): δ0.78 (3H, *s*, H-14), 1.22 (3H, *d*, J = 7 Hz, H-13), 1.61 (3H, *s*, H-15), 5.33 (1H, *m*, H-3).

Lithium–ammonia reduction of **1a**. A soln of **1a** (100 mg) in THF (3 ml) was added to a stirring soln of Li (50 mg) in liquid NH_3 (10 ml) under N_2 at −40°, and the mixture was then stirred for 30 min. Work-up and purification as above, gave **7** (68 mg) (for analytical and spectroscopic data *vide supra*).

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