CONSTITUENTS OF HELIANTHUS CILIARIS*

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Abstract—A new diterpene, 7-a-hydroxy-4-epitrachylobanic acid, has been isolated from Helianthus ciliaris DC No sesquiterpene lactones were found in this plant

INTRODUCTION

FURTHER study of plants of the tribe Heliantheae (Compositae) has been extended to a Helianthus species in a search for sesquiterpenoid lactores. None of these was obtained, but a compound, ciliaric acid, was isolated which proved to be a new pentacyclic diterpene of the trachylobane class.

RESULTS AND DISCUSSION

Charic acid (I) was obtained in yields of 0.01 and 0.02% from two collections of H. ciliaris \ddagger The compound, C₂₀H₃₀O₃, had m.p. 304-305°. Its IR spectrum showed the presence of a carboxyl group (1690 cm⁻¹) and hydroxyl groups (3600 and 3400 (broad) cm⁻¹) The mass spectrum showed the molecular ion at m/e 318, a prominent ion at m/e285 (M—CH₃—H₂O) and the base peak at m/e 300 (M—H₂O). There was no significant absorption in the UV.

The NMR spectrum of I showed three 3-proton singlets at δ 1 34, 1.22 and 1 16, indicating the presence of three tertiary methyl groups A one-proton multiplet at δ 3 64 shifted to δ 4 85 in the monoacetate (II), it consisted of a pair of doublets (J = 10, 6 Hz) in II, and is assigned to the proton of CHOAc at C-7 The coupling pattern indicates that the C-7 hydroxyl group is α and equatorial The presence of the cyclopropyl grouping in I and all of its derivatives was disclosed by broad multiplets between δ 0 5 and 0 8 in their NMR spectra A similar signal is observed for the cyclopropyl protons of trachylobanic acid, as



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a broad multiplet between δ 0.4 and 0.8,¹ and the corresponding cyclopropyl protons of ishwarone (XI) appear as a broad multiplet centered at δ 0.5²

Ciliaric acid (I) was not hydrogenated with platinum in acetic acid and was recovered unchanged after treatment with *m*-chloroperbenzoic acid for 24 hr. Further, the methyl ester (III) of I showed no color with tetranitromethane and decolorized neither cold, dilute potassium permanganate nor bromine in carbon tetrachloride These observations, coupled with the fact that no proton signals were present in the vinyl region of the NMR spectrum, indicate that ciliaric acid contains no carbon-carbon double bond and is pentacyclic.

Oxidation of the methyl ester (III) with Jones' reagent yielded the corresponding ketone (IV). The 60 MHz and 100 MHz NMR spectra of this compound are in complete accord with the assigned structure and further indicate the location of the carbonyl group at C-7. The AB portion of an ABX pattern can be clearly discerned, the C-6 α proton appearing as a pair of doublets (J = 16, 14 Hz) centered at δ 2 93.

Additional information could be gained from examination of the NMR spectra of IV. The 100-MHz spectrum shows a one-proton doublet centered at $\delta 2.32$ (J = 12 Hz, geminal). This signal, also discernible in the 60-MHz spectrum, can be assigned to one of the C-15 protons An examination of models shows that this proton is in sufficiently close proximity to the carbonyl group at C-7 to suffer proximity deshielding and to separate its signal from the bulk of the skeletal protons at higher fields There is ample precedent for this interpretation. The C-1 β proton of 5 α -androstan-11-one is deshielded by the C-11 carbonyl group, and its signal is seen at $\delta 2.45$. Similarly, the signal for the C-15 α proton in 5 α -androstan-7-one appears at $\delta 2.26.3$

The relative positions of the carboxyl (C-4) and hydroxyl (C-7) groups are supported by chemical as well as spectral evidence. Charic acid (I) could not be lactonized by treatment with *p*-toluenesulfonic acid The keto acid (V) corresponding to I melted at 209–210° without decarboxylation This is in contrast to the corresponding 3-hydroxy acid, which loses carbon dioxide readily upon oxidation to the β -keto acid¹



The position and orientation of the carboxyl group can be inferred from spectral data. Bory and Fétizon have examined a series of di- and triterpene methyl esters and observed that compounds with the partial structure VI show strong IR absorption at 1155 $\pm 4 \text{ cm}^{-1}$, while those having the partial structure VII show an absorption band at 1244 $\pm 4 \text{ cm}^{-1}$.⁴ The IR spectra of the ciliaric acid methyl ester (III), the 7-keto ester (IV) and the 7-deoxy ester (IX) all show prominent absorption bands at 1150 cm⁻¹, and thus correspond with structure VI (but of opposite configuration to that shown).

¹ G HUGEL, L LODS, J M MELLOR, D W THEOBALD and G. OURISSON, Bull Soc Chim Fr 2882 (1965)

² T R GOVINDACHARI, K NAGARAJAN and P C PARASARATHY, Chem Commun 823 (1969)

³ N S BHACCA and D. H. WILLIAMS, Applications of NMR Spectroscopy in Organic Chemistry, Chap 4, Holden-Day, San Francisco (1964)

⁴ S BORY and M FÉTIZON, Bull Soc Chim Fr 570 (1964)

Narayanan and Venkatasubramanian⁵ have described the effects of position and stereochemistry of carboxylic acids and the corresponding esters upon the chemical shifts of tertiary methyl groups in a series of diterpenes. They observed that a methyl group in a 1,3-diaxial relationship to a methoxycarbonyl group is deshielded by about 0.28 ppm when —COOMe is changed to —COOH (spectra in pyridine). The corresponding spectra of C-4 equatorial acids show only a slight (0 07 ppm) alteration of the chemical shift of the C-10 methyl group when this change is made. Moreover, it was found that when the NMR spectra of the C-4 acid and its ester are taken in pyridine solution, the C-4 methyl group is deshielded about 0.17 ppm in the acid, relative to the ester. It was further noted that the signal for the C-10 methyl group in diterpene 4-carboxylic esters is found at 0.22 ppm higher field in chloroform (and at 0 10 ppm higher field in pyridine) when —COOMe is axial than when —COOMe is equatorial.

C-4/C-7		in Pyridine-d.			in Chloroform-d		
		C-4 Me	C-16 Me	C-10 Me	C-4 Me	C-16 Me	C-10 Me
C-4 COOH (a) C-7 OH (e)	(1)	1.34	1 16	1 22			
C-4 COOMe (a C-7 OH (e)) (III)	1.21	1 16	0 90	1 15	1 15	0 79
C-4 COOH (a) C-7 OAc (e)	(II)	1 29	1 13	1 13	1 23	1 13	0 93
C-4 COOMe (a C-7 C == 0	.) (TV)				1 19	1 15	0 95
C-4 COOH (a) C-7 CH₂	(VIII)	1 34	1.16	1 15	1 20	1 14	0 88
C-4 COOMe (a C-7 CH₂) (IX)	1 17	1 17	0 84	1 12	1 12	0 77
C-4 COOMe (e C-7 CH₂) (X)	1 21	1 15	0-91	1 12	1 12	0 95

TABLE 1 CHEMICAL SHIFTS (δ)

The chemical shifts observed for ciliaric acid and its derivatives are in complete accord with these observations if the —COOH group is axially (a) disposed. The relevant data are given in Table 1. It is seen that the signal of the C-10 methyl group is shifted 0.32 ppm from its position (1 22) in the acid (I) to that (0.90) in the spectrum of the ester (III). Furthermore, the signals of C-4 Me and C-10 Me are shifted by 0 17 and 0 31 ppm, respectively, ⁵ C. R NARAYANAN and N K. VENKATASUBRAMANIAN, *Tetrahedron Letters* 3639 (1965).

from their positions in the spectra of VIII (C-4 Me, 1.34; C-10 Me, 1.15) to 1.17 (C-4 Me) and 0.84 (C-10 Me) in the spectrum of IX. The predicted⁵ values of these differences are 0.17 and 0.28 ppm for the shielding effects of axial C-4 COOH upon C-4 Me and C-10 Me, respectively.

Further examination of the data of Table 1 reveals that the differences in chemical shifts of the C-10 methyl groups of IX and X are also in accord with the results of the model studies ⁵ Thus, the signals for C-10 Me of IX and X appear at $\delta 0.84$ and $\delta 0.91$ (difference, 0.07) respectively in pyridine, and at 0.77 and 0.95 (difference, 0.18) in chloroform. These differences are in good agreement with the expected values of 0.10 in pyridine and 0.22 in chloroform



FIG 1 MASS SPECTRUM OF 4-EPITRACHYLOBANIC ACID METHYL ESTER (IX)

The methyl ester of the deoxy compound (IX) was different from trachylobanic acid methyl ester (X).* A mixed m p. showed a marked depression, and although the IR spectrum of IX and X are similar in most respects, that of the latter showed an absorption band at 1250 cm⁻¹, in accord with the observations described above ⁴ The most striking evidence of the close similarity of IX and X was found in the mass spectra. These are shown in Figs 1 and 2, and it can be seen that they are nearly identical in all details of the cracking pattern. The near identity of the spectra indicate that initial fragmentations of the epimers lead at once to identical ions which then follow identical courses of further fragmentation. The appearance in both mass spectra of ions at m/e 301 (M-15) and 257 (M-59) suggest that an



FIG 2 MASS SPECTRUM OF TRACHYLOBANIC ACID METHYL ESTER (X)

* We are grateful to Professor G Ourisson for a specimen of trachylobanic acid methyl ester (X)



early fragmentation leading to the loss of the C-4 methyl and methoxy carbonyl group occurs; this would lead to the same initial ions (XII) and (XIII):

The absolute configuration of ciliaric acid can be inferred from the optical rotations of IX and X The specific rotation of the former is $[a]_{D}^{25} -62^{\circ}$ (c 13·4, CHCl₃), and of the latter $[a]_{D}^{25} -41^{\circ}$ (c 20, CHCl₃). Since these values are of the same sign and comparable magnitudes it is unlikely that the compounds differ at centers other than at C-4. The occurrence in nature of terpenoid components differing only in the epimeric relationship of --CH₃ and --COOH at C-4 is not unprecedented Two other such pairs are kaurenoic (4-a-COOH; $[a]_{D} -110^{\circ}$) and kaurenic (4- β -COOH; $[a]_{D} -61^{\circ}$) acids; and vinhaticoic (4-a-COOH; $[a]_{D} +73^{\circ}$) and voacapenic (4- β -COOH; $[a]_{D} +108^{\circ}$) acids It is to be noted that the relative optical rotatory powers of deoxyciliaric and trachylobanic acids are consistent with these.

Note added in proof The occurrence of the alcohol (CH₂OH for COOH) corresponding to compound VIII in *Helianthus annuus* has recently been reported (O ACHMANTOWICZ, A EJCHART, J JURCZAK, L KO-ZERSKI and J ST PYREK, *Chem Commun* 98 (1971)

EXPERIMENTAL

Extraction of plant material Dried and ground *Helianthus ciliaris* DC (RJB-68361) (4 kg) was extracted with CHCl₃ Removal of the solvent left 220 g of crude extract, which was partitioned between 1 5 1 of hexane and 1 5 1 of MeOH-H₂O (3 1) Each layer was reextracted with 500 ml of the opposite phase Examination of the extracts by TLC showed that the hexane phase contained none of the components of the alcoholic solution The latter was extracted with CHCl₃, from which was obtained 55 g of a brown oil Chromatography of this material on 800 g of silica gel with CHCl₃ containing increasing proportions of acetone (25 1-1 fractions) was monitored by TLC Fractions 17–21 showed a single component, they were combined and evaporated to a volume of about 25 ml From this solution 0 80 g of ciliaric acid (I) separated as a white powdery solid A second extraction of 5 kg of another collection of plant (RJB-69322) yielded 0 40 g of I

7-a-Hydroxy-4-epitrachylobanic acid (ciliaric acid) (I) The crude material from the chromatographic separation was recrystallized from ethanol to give colorless spears, m p $304-305^{\circ}$ (Found C, 75 52, H, 9 53 C₂₀H₃₀O₃ required C, 75 43, H, 9 50%) The spectral properties are described in the Discussion

7-a-Acetoxy-4-epitrachylobanic acid (II) Acetylation of I with Ac₂O-pyridine yielded the acetate Recrystallized from aqueous ethanol, it formed colorless prisms, m p 228-230° The mass spectrum showed a molecular ion peak at m/e 360, a base peak at m/e 300 (M-HOAc), and a prominent peak at m/e 285 (M-HOAc-CH₃) The NMR spectrum (pyridine-d₅) showed three singlets at 1 13 (6H, C-10 CH₃, C-16 CH₃), 1 29 (3H, C-4 CH₃) and 2 02 (3H, OAc), and a broadened pair of doublets (1H) at δ 4 85 (J = 10, 6 Hz) (Found C, 73 56, H, 8 86 C₂₂H₃₂O₄ required C, 73 30, H, 8 95%)

7-a-Hydroxy-4-epitrachylobanic acid methyl ester (III) Esterification of ciliaric acid with CH_2N_2 (in ether) afforded the methyl ester, colorless prisms from ligroin, m p 136–137° The mass spectrum showed no molecular ion, but a principal peak at m/e 299 (M-H₂O-CH₃) The IR spectrum showed peaks at 3600 (OH) and 1720 (COOMe) cm⁻¹ There was no absorption in the UV region The NMR spectrum (CDCl₃) showed signals at δ 3 60 (3H, OCH₃) and a quartet at δ 3 32 (1H, J = 11, 4 Hz, C-7 H), besides the signals for the three methyl groups (Found C, 75 83, H, 9 70 C₂₁H₃₂O₃ required C, 75 86, H, 9 70%)

7-Oxo-4-epitrachylobanic acid methyl ester (IV) A solution of 70 mg of the methyl ester (III) of ciliaric acid was treated at 0° with 1 ml of Jones' reagent The product, isolated by CHCl₃ extraction, crystallized from ligroin as colorless platelets, m p 94-96° Its IR spectrum showed a strong band at 1720 cm⁻¹ (COOMe and C-7 C=O) The NMR spectrum showed the OCH₃ signal at δ 3 65, in addition to the features described in the Discussion Mass determination Found 330 2194 \pm 0 002 Calc for C₂₁H₃₀O₃ 330 21948

7-Oxo-4-epitrachylobanic acid (V) In a first attempt to reduce the C-7 carbonyl group of IV with hydrazine hydrate and KOH, the product proved to be the acid derived by simple saponification of the ester The acid (V) crystallized from ethanol as colorless spears, mp 209-210°. It showed IR absorption at 3400 (COOH), 1720 (C=O) and 1690 (COOH) cm⁻¹ The mass spectrum showed the molecular ion peak at m/e 316, along with a prominent ion at m/e 301 (M-CH₃) It is significant that there was no indication that the loss of CO₂ was more facile than in the fragmentation patterns of those derivatives lacking the carbonyl group Mass determination⁻ Found 316 2038 \pm 0 003, Calc for C₂₀H₂₈O₃ 316 20383

4-Epitrachylobanic acid (VIII) Repetition of the Wolff-Kishner reduction of (IV) under more vigorous conditions yielded the 7-deoxy acid, m p $126-129^{\circ}$ Its IR spectrum showed absorption at 3400 and 1690 cm⁻¹ (COOH), and the mass spectrum showed the molecular ion at m/e 302 and a prominent peak at m/e 287 (M-CH₃) The compound was not further characterized, except for the NMR spectrum (Table 1), but was converted into the methyl ester

4-Epitrachylobanic acid methyl ester (IX) Esterification of VIII with CH_2N_2 in ether afforded the methyl ester It crystallized from petroleum as white leaflets, m p 114-116° It showed IR absorption at 1720 cm⁻¹ The NMR spectrum is given (in part) in Table 1, the mass spectrum of IX in Fig 1, and that of X in Fig 2. Mass determination Found 316 24038 \pm 0 0003, Calc for $C_{21}H_{32}O_2$ 316 240217

An authentic specimen of trachylobanic acid methyl ester $(X)^9$ had m p 110-112°, and a mixture of IX and X melted at about 96-100°

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Key Word Index—Helianthus ciliaris, Compositae, pentacyclic diterpene, terpene, 7-a-hydroxy-4epitrachylobanic acid