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New Antibacterial Tetratriacontanol Derivatives From *Agave americana* L.

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ABSTRACT - *Three biogenetically related compounds have been isolated from Agave americana L. namely 5-hydroxy-7-methoxy-2-tritriacontyl-4H-1-benzopyran-4-one, tetratriacontyl hexadecanoate and tetratriacontanol. Two of them exhibited significant antibacterial activity.*

INTRODUCTION

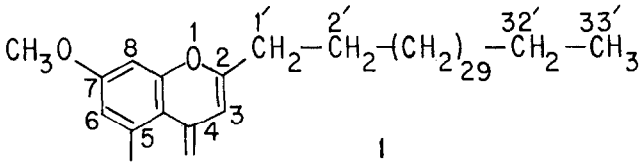
We undertook the chemical investigation of *Agave americana* L. because of its traditional use as drug in the Indian system of medicine (used as diuretic, antisyphilitic, laxative, emmenagogue and antiscorbutic).[1-3] Not much phytochemical work is reported on this plant and the compounds previously isolated belong to the class of spirostanol glycosides,[4] saponins[5-7] and steroidal saponins.[8-9] In this paper we report the isolation and biological activity of three compounds from the combined petrol and benzene extracts of *Agave americana* L.

RESULTS AND DISCUSSION

The column chromatographic separation of the concentrate of petrol and benzene extracts of the air-dried aerial part of *Agave americana* L. led to the isolation of three compounds: **1**,**2** and **3**. The structure elucidation and antibacterial activity studies of these compounds are described below.

Compound 1 crystallised as colourless needles from petroleum ether-chloroform mixture, m.p. 83°C and analysed for $C_{43}H_{74}O_4$ which is also corroborated by the M^+ peak at m/z 654 in its mass spectrum. Both the UV and IR absorption maxima [251, 286(sh)nm and 2900, 2825, 1658, 1615 and 1560 cm^{-1}, respectively] of the compound were typical of a chromone nucleus.[10] The presence of a chelated phenolic hydroxyl group in the compound was indicated by its characteristic colour reaction (violet) with alcoholic ferric chloride, a bathochromic shift of 50 nm in its λ max in the presence of $AlCl_3$/HCl and by a singlet at δ 12.71 in its ^{1}H-NMR spectrum. Other signals in its ^{1}H-NMR spectrum were a singlet at δ 3.84 integrating for 3 protons and two *meta*-coupled doublets at δ 6.32 and 6.34 (J=3Hz each). This indicated that the benzenoid ring of the chromone moiety is carrying a -OH group and a $-OCH_3$ group at the C-5 and C-7 positions, respectively. The presence of a thirty three carbon saturated chain was indicated in the molecule by its ^{1}H-NMR spectrum as a triplet at δ 0.90,

δ 1.25, a multiplet at δ 1.73 and a triplet at δ 2.56 appeared for 3H, 60H, 2H and 2H, respectively. The presence of a singlet at δ 6.01 for one proton indicated that the C-3 position of the chromone is unsubstituted, thus indicating that the tritriacontyl side chain is located at the C-2 position. Thus on the basis of the above spectral data, we propose the structure: 5-hydroxy-7-methoxy-2-tritriacontyl-4H-1-benzopyran-4-one to compound **1**.



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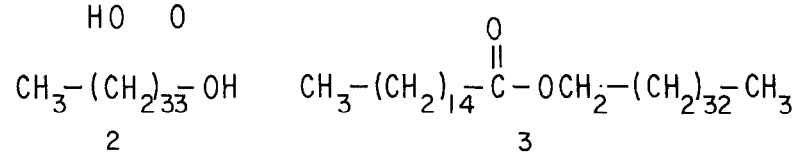

2

3

The ^{13}C-NMR and mass spectra of compound **1** are also in agreement with the proposed structure. Thus, its ^{13}C-NMR spectrum showed the characteristic peaks of carbonyl in the pyran ring at δ 185.5, other peaks for ring carbons were at δ 164.5 (C-7), 149.68 (C-5 and C-8a), 144.0 (C-2), 107.9 (C-3 and C-4a), 97.7(C-8), 92.3(C-6) and the methoxy group appeared at 55.6 ppm. The carbons of the tritriacontyl group were spread over δ 13.9 to 34.11 ppm. In the MS of compound **1**, the molecular ion peak was observed at m/z 654; other significant fragmentation peaks were at m/z 626, 219, 206 and 167. The peaks due to consecutive loss of -CH_2- or -CH_2-CH_2- at 14 and 28 mass unit differences and characterstic peaks at 43 (C_3H_7), 57 (C_4H_9) and 71 (C_5H_{11}), due to the presence of long chain alkyl group in the molecule were also observed.

The highest alkyl chain at the C-2 position among natural chromones has so far been hentriacontyl (C_{31}), Cooke and Down [11] have isolated an inseparable mixture of C_{27}-, C_{29}- and C_{31}- alkylated chromones from *Dianella revoluta* and *Stypandra grandis*. Thus far the longest alkyl chain (C_{15}) bearing chromone isolated in the pure form has been 5,7-dihydroxy-2-pentadecylchromone from the seaweed *Zonaria tournefortii*.[12] We for the first time report the isolation of chromone **1** carrying the C_{33} alkyl chain at the C-2 position in the pure form. It is a new compound as it has neither been isolated from any natural source nor synthesised earlier. Our sample of compound **1** exhibited strong antibacterial activity.

Compound 2 crystallised as a colourless waxy solid from petroleum ether-chloroform mixture, m.p. 81°C. It analysed for $C_{34}H_{70}O$ which is corroborated by its mass spectrum. The characterstic mass fragmentation peaks due to loss of consecutive -CH_2- units (14 mass unit) in its mass spectrum indicated that it possesses a long aliphatic chain. From the strong M-18 peak in its mass spectrum and characteristic peaks in the ^{1}H-NMR spectrum, compound **2** was deduced to be a C_{34}- saturated primary alcohol, i.e. tetratriacontanol (**2**). It showed a triplet at δ 3.64 due to 2 methylene protons (-$C\underline{H}_2$OH), a pentat at 1.60 (2H), a broad singlet at δ 1.25 integrating for 62 protons and a high field triplet at δ 0.88 due to terminal -CH_3 group. The ^{13}C-NMR spectrum of compound **2** also supports this structure, which showed characterstic peaks at δ 63.02 and 32.7 due to O-$\underline{C}$ and O-C-$\underline{C}$, cluster of peaks at δ 29.5 and peaks at δ 21.5 and 13.9 due to the terminal carbon atoms. This alcohol has earlier been isolated as a mixture with tritriacontanol, dotriacontanol, hentriacontanol and triacontanol from *Strobilanthes callosus*.[13] We have isolated the alcohol **2** in the pure form and recorded its complete spectral data for the first time.

Compound 3 crystallised as colourless flakes from petrol-chloroform mixture, m.p. 71 °C. It analysed for $C_{50}H_{100}O_2$, which is corroborated by the M^+ peak at m/z 732 in its mass spectrum. The mass spectral fragmentation pattern along with other spectral data indicates that compound **3** is an ester of a C_{16} saturated acid and the C_{34} saturated primary alcohol moeity. In its mass spectrum, it showed the base peak at m/z 257 due to cleavage at the O-C bond and hydrogen migration. The ^{1}H-NMR spectrum showed two triplets at δ 4.02 and 2.28(2H each) due

to $COOCH_2$- and -CH_2-COO, respectively, a pentat at δ 1.58 for 2H due to-O-CH_2-CH_2-, a broad singlet at δ 1.25 integrating for 88 protons due to the 44 methylene groups of acid and alcohol moieties and a high field triplet at δ 0.9 (6H) due to the two terminal -CH_3 groups of acid and alcohol residues.The characteristic peaks in its ^{13}C-NMR spectrum which appeared at δ 173.86 (-COO-), 64.28(-OCH_2-), 34.33 (-CH_2-COO-), 31.83 (-O-CH_2-CH_2) also supported the structure. On the basis of above spectral analysis, structure of compound 3 was deduced as tetratriacontyl hexadecanoate, which is found to be a new compound.

The above three compounds, 2-tritriacontyl-5-hydroxy-7-methoxychromone (1), tetratriacontanol (2) and tetratriacontyl hexadecanoate (3) are apparently biogenetically related to each other. The presence of hexadecanoic acid (palmitic acid) in natural sources is well known. Tetratriacontanol (2) is also known to be a natural product and has been isolated by us from *A. americana*. Therefore, biogenetically one can expect in *A. americana* the presence of tetratriacontyl hexadecanoate (3) which we have isolated for the first time. Further the basic skeleton of the long n-alkyl chromone 1 could arise from four malonyl CoA units via contanoyl CoA as starter. Thus, the presence and isolation of ester 3 and chromone 1 from *A. americana* L. is probably biogenetically related.

Biological Activity of Compounds 1, 2 and 3

Antibacterial activity of the three compounds isolated from *A. americana* has been tested against four bacteria (Table I) and it was found that 2-tritriacontyl-5-hydroxy-7-methoxychromone is highly active against *Pseudomonas aeruginosa* and tetratriacontanol (2) shows fairly good activity against *Staphylococcus faecalis*; no appreciable activity is shown by 3 against any of the four bacteria used in our experiments.

TABLE - I Antibacterial activity of compounds isolated from *A. americana*

Bacteria	2-Tritriacontyl-5-hydroxy-7-methoxychromone (1)	Tetratriacontanol (2)	Tetratriacontyl hexadecanoate (3)
Escherichia coli	*(++)*	*(-)*	*(-)*
Pseudomonas aeruginosa	*(+++)*	*(-)*	*(+)*
Staphylococcus aureus	*(-)*	*(+)*	*(-)*
Staphylococcus faecalis	*(-)*	*(++)*	*(-)*

EXPERIMENTAL

The m.pts. were taken in H_2SO_4 bath and are uncorrected. The UV spectra were measured in spectroscopic grade MeOH on a Beckman model no. 26 spectrophotometer and IR spectra were recorded in KBr discs on a Beckman model Acculab spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker AC-250 instrument at 250 and 62.5 MHz, respectively with dry TMS as the internal standard. The chemical shifts are expressed in ppm downfield from TMS on δ scale. The mass spectra were recorded on a Varian mat 311A instrument. The silica gel (60-80 mesh) was used for column chromatography and silica gel G for TLC. All analytical samples were routinely dried over P_2O_5 in vacuo and were tested for purity by TLC. Dry Na_2SO_4 was used for drying organic solvents and petrol used had b.p. 60-80°C.

Isolation of compounds. Air- dried aerial part (4 kg) of *Agave americana* was extracted with petroleum ether and benzene in succession in a soxhlet apparatus for 60 hrs each. The two extracts were examined on TLC in a number of solvent systems and found to be similar. These two extracts were combined, concentrated and the residue chromatographed which lead to the isolation of three compounds from the eluent petroleum ether-chloroform (19:1) in the order **3**, **1** and **2**.

5-hydroxy-7-methoxy-2-tritriacontyl-4H-1-benzopyran-4-one (**1**), colourless crystals, m.p. 83°C (petroleum ether-ethyl acetate); R_f=0.45 (petroleum ether-benzene, 1:1); UV λ max: 251, 286(sh)nm; +$AlCl_3$/HCl: 300, 360 nm; IR ν max: 2900, 2825, 1658, 1615, 1560, 1500 and 1350 cm^{-1}; ^{1}H-NMR : δ 0.90 (3H, t, J= 8 Hz, -CH_3), 1.25 (60 H, br s, -$(CH_2)_{30}$-CH_3) , 1.73 (2H, m, -2′CH_2), 2.56 (2H, t, J=8Hz, -1′CH_2), 3.84 (3H, s, -OCH_3), 6.01 (1H, s, H-3), 6.32 (1H, d, J=3 Hz, H-6), 6.34 (1H, d, J=3Hz, H-8) and 12.71 (1H, s, chelated -OH); ^{13}C-NMR : δ 13.9(C-33′), 22.5(C-32′), 26.7-29.6(C-3′ to C-31′), 31.83(C-2′), 34.11(C-1′), 55.6(OCH_3), 92.3(C-6), 97.7(C-8), 107.9(C-3 and C-4a), 144.0(C-2), 149.68(C-5 and C-8a), 164.5(C-7) and 185.5(C-4); EIMS m/z(rel. int.) : 654[M^+] (9), 640 (M^+-CH_2) (14), 626(M^+-CO) (100), 598(10), 429(16), 387(6), 359(7), 321(12), 288(10), 219(53), 206(20), 167(10), 71(5),57 (12), 43(12) and 39(3).

Tetratriacontanol (**2**) white waxy solid, m.p. 81° C (petroleum ether - chloroform); R_f = 0.36 (benzene); IR ν max 3350 ; ^{1}H-NMR: δ 0.88 (3H, t, J=7 Hz, terminal -CH_3), 1.25 (62H, br s, $CH_3(CH_2)_{31}CH_2CH_2$), 1.60 (2H, m, -OCH_2-CH_2-) and 3.64 (2H, t, J=7Hz, -CH_2-OH) ; ^{13}C-NMR: δ 13.9 (C-34), 21.5 (C-33), 29.5 (C-3 to C-32), 32.7 (C-2) and 63.02 (C-1); EIMS (m/z) (rel. int.): 476 [M^+-18] (14), 448(11), 420(4), 392(1), 125(18), 111(34), 97(68), 85(34), 83(78), 71(58), 57(100), 43(68) and 18(8).

Tetratriacontyl hexadecanoate (**3**), colourless flakes, m.p. 71° C (petroleum ether); R_f = 0.30 (petroleum ether- benzene 49:1); IR ν max: 3400, 2900, 2838, 1730, 1610, 1460 and 1165 cm^{-1}; ^{1}H-NMR: δ 0.9 (6H, t, J = 7 Hz, two terminal -CH_3), 1.25 [88 H, br s, -$OCH_2(CH_2)_{32}CH_3$ of alcohol and $CH_3(CH_2)_{12}CH_2CH_2$-CO- of acid moities], 1.58 (2H, m, -CH_2-CH_2-CO), 2.28 (2H, t, J=7Hz, -CH_2-CO) and 4.02 (2H, t, J=7Hz, -OCH_2-) ; ^{13}C-NMR: δ 13.99 (two terminal -CH_3), 22.59 to 30.90 (C- 3 to C- 33 of alcohol and C- 3 to C- 15 of acid moieties), 31.83(O-CH_2-CH_2-), 34.33 (-C-CO), 64 .28 (C-O-CO) and 173.86 (-COO-) ; EIMS (m/z) (rel. int.): 732 [M^+](12), 704(26), 676(22), 648(16), 493(2), 475(2), 448(6), 420(5), 341(6), 285(14), 257(100), 240(8), 212(4), 111(24), 83(50), 71(60), 57(92) and 43(60).

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