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GUGGULU (RESIN FROM *COMMIPHORA MUKUL*)—3 LONG-CHAIN ALIPHATIC TETROLS, A NEW CLASS OF NATURALLY OCCURRING LIPIDS†

V. D. PATIL, U. R. NAYAK and SUKH DEV
National Chemical Laboratory, Poona 8, India

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Abstract—Isolation and structure elucidation of two long-chain aliphatic tetrols, now formulated as octadecan-1,2,3,4-tetrol and eicosan-1,2,3,4-tetrol, from gum-resin of *Commiphora mukul*, is described. There is evidence for the occurrence of nonadecan-1,2,3,4-tetrol, as well, in the resin. This is the first reported occurrence of such compounds in nature, though the closely related phytosphingosines (e.g. 2-amino-octadecan-1,3,4-triol) are well known.

It has been reported[1] that, the ethyl acetate extract of the gum-resin exudate from the tree *Commiphora mukul* (Hook, ex Stocks) Engl. (Syn. *Balsamodendron mukul* Hook, ex Stocks) on hydrolysis, furnishes a complex mixture of neutral products which can be partially resolved by systematic chromatography on SiO_2-gel to give several steroids. The more polar fractions (eluted with 2–50% MeOH in EtOAc), from this chromatography, on rechromatography, furnished a product which after several recrystallisations from acetone yielded colourless prismatic needles, m.p. 73–77° (overall yield ~2% on gum-resin). This product has now been characterised‡ as a mixture of octadecan-1,2,3,4-tetrol (~50%), nonadecan-1,2,3,4-tetrol (~7%) and eicosan-1,2,3,4-tetrol (~40%) with minor amounts of other components, possibly lower (C_{16}, C_{17}) and higher (C_{21}, C_{22}) homologous tetrols.

That the above product (m.p. 73–77°) is a mixture of homologs, became evident while studying the electron-impact-induced fragmentation of this material and its derivatives. Though this mixture could be partly resolved on TLC over SiO_2-gel impregnated with boric acid,[2] preparative chromatography (PLC, inverted-dry-column-chromatography) proved futile. Once the structure became apparent, separation could be achieved by GLC of the derived acetonides and, this is discussed later. It was found expedient to carry out structural investigations on the homologous mixture and this sufficed to unravel the structures of the components.

The product (m.p. 73–77°) shows in its IR spectrum strong OH absorption (3400, 3180, 1075 and 1022 cm^{-1}), but no C=O absorption. It gives no color with tetranitromethane (absence of C=C bonds). It readily underwent complete acetylation (Ac_2O, pyridine) at room temp. (~25°, 16 hr) to furnish a crystalline acetate (m.p. 42–43°. IR: OAc 1750 and 1230 cm^{-1}; no OH band) showing a PMR spectrum typical of long-chain aliphatic derivatives; PMR (CCl_4): $CH_3{\cdot}CH_2$— (3H, t, 0·88 ppm, $J = 6$ Hz), —$(CH_2)_n$— (~26H, 1·28 ppm), $OCOCH_3$ (~11H, essentially a singlet, 2·07 ppm), —CH_2OAc (~2H, m, 3·63–4·53 ppm) and CHOAc (~3H, m, 4·80–5·33 ppm); when the spectrum was taken in C_6H_6, the acetate signal clearly split into four ~3H singlets at 1·723, 1·733, 1·750 and 1·775 ppm. These data suggested that the product is a tetrol (one primary OH and three sec OH) having a long aliphatic chain. This conclusion received full support from the PMR spectrum (CCl_4) of the derived formate (m.p. 68–70°. IR: OCOH 1740, 1180 and 1150 cm^{-1}), which shows four ~1H singlets at 7·916, 7·950, 7·983 and 8·013 ppm, assignable to OCOH protons.

The mass spectrum of the formate§ helped in arriving at the size of the molecular species present and, further enabled us to locate the positions of the hydroxyls. Three homologous species are discernible in the mass spectrum of the formate.

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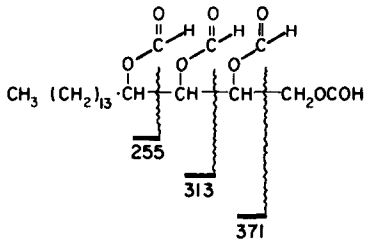
‡In the earlier communication[1] this material has been incorrectly reported as a mixture of long-chain aliphatic triols. This error arose from the difficulty in getting the correct mol. wt. from mass spectra and, at that time we considered the fourth oxygen atom to be located as an oxirane ring.

§Mass spectra of the free tetrol and the tetra-acetate were not helpful in determining the mol. wt. of the species present. The tetrols always gave highest *m/e* peak corresponding to $M + 1^+$ and this proved quite confusing in the early stages of the work; mass spectra of the pure tetrols are discussed later. Similarly, the mass spectrum of the tetra-acetate was also not simple to interpret.



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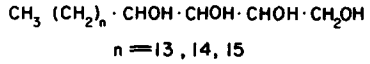

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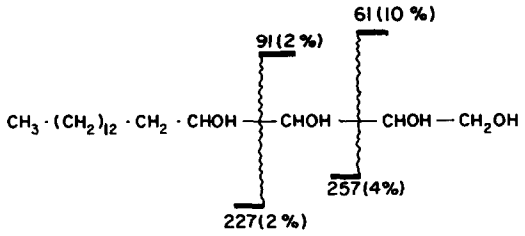
Thus, at the highest mass, ion *m/e* 412 is present, which is considered to be $(M\text{-}HCOOH)^+$, ion at *m/e* 366, 320 and 274 correspond respectively to $(M\text{-}2HCOOH)^+$, $(M\text{-}3HCOOH)^+$ and $(M\text{-}4HCOOH)^+$ (relative intensities of the four ions: *m/e* 412:366:320:274::3·4:1:5). The highest mol. wt. tetrol species is, thus, considered to be $C_{20}H_{42}O_4$ (mol. wt. 346; mol. wt. of tetra-formate, 458). Two lower homologous series beginning with *m/e* 398 and 384 (tetraformates —HCOOH) are also present and represent tetrols $C_{19}H_{40}O_4$ and $C_{18}H_{38}O_4$ respectively. Elemental analysis is also consistent with $C_{19}H_{40}O_4 \pm CH_2$. That the four hydroxyls must be located on C_1, C_2, C_3 and C_4, bacame clear from the mass spectrum of the formate, which shows ions at *m/e* 255, 313 and 371 (relative intensities: *m/e* 371:313:255::1:5:15), corresponding to the fragmentation shown in 1 for the formate derived from $C_{18}H_{38}O_4$; corresponding ions for the C_{19} and C_{20} series are also present. Thus, the tetrol (m.p. 73–77°) is considered to be a mixture of the C_{18}, C_{19}, C_{20} homologs with the structure 2 (the percentage composition is discussed below).

Chemical evidence in favour of 2 was readily forthcoming. On quantitative periodate oxidation it was found that the product (m.p. 73–77°) consumed[3] three mole equivalents of periodic acid and, in the process ~2 mole equivalents of formic acid[4] were formed. These results are fully consistent with the hydroxyl substitution pattern, as shown in 2. The long-chain aliphatic moiety in the periodate oxidation was trapped as its 2,4-dinitrophenylhydrazone (m.p. 88–90°). Its PMR spectrum confirmed that the product is a derivative of aldehyde(s) (1H, t, 7·5 ppm, $J = 6$ Hz)[5] and its mass spectrum clearly showed ions at *m/e* 434 (highest mass), 420 and 406 corresponding to the parent aldehydes $C_{15}H_{30}O$, $C_{16}H_{32}O$ and $C_{17}H_{34}O$ respectively.

Next, it was desired to quantitatively determine the composition of the tetrol mixture and to isolate the major components in a state of purity. The tetrol mixture (m.p. 73–77°) readily furnished a bis-acetonide, which was found to be readily amenable to GLC separation; in contrast, the GLC of the acetates or formates failed to give complete separation. GLC of the acetonide showed it to consist of at least seven components with RRT (% present) of 1 (0·7%), 1·35 (1·8%), 1·65 (47·8%), 2·24 (7·2%), 2·71 (39·8%), 3·59 (0·2%) and 4·35 (2·4%). The two major components (RRT: 1·65, 2·71) were separated by preparative GLC and identified by mass spectrometry as the bis-acetonides based on C_{18} tetrol (RRT, 1·65; M^+ *m/e* 398) and C_{20} tetrol (RRT 2·71; M^+, *m/e* 426). Taking into consideration the results of the spectroscopic and chemical studies discussed earlier, the third major component (RRT 2·24, 7·2%) must be the acetonide derived from the tetrol, $C_{19}H_{40}O_4$. The remaining four, very minor constituents, are considered to be the other homologous members (C_{16}, C_{17}, C_{21}, C_{22}). This is supported by plot of the log of the retention times *vs* the number of C atoms (of the aliphatic chain), when all the points fall nicely on a straight line.[6]

The two pure acetonides, on mild acid hydrolysis (10% $HClO_4$ aq-dioxane) regenerated the parent tetrols, which were purified by crystallisation: $C_{18}H_{38}O_4$ (m.p. 80–82°, $[\alpha]_D + 11{\cdot}4°$), $C_{20}H_{42}O_4$ (m.p. 85–87°, $[\alpha]_D + 9{\cdot}6°$). Both of these pure tetrols do not show in their mass spectra, M^+ ion, but instead, $M+1^+$ is exhibited (C_{18}, *m/e* 319, 0·07%; C_{19}, *m/e* 347, 0·1%); the occurrence of $M+1^+$ ion (arising from ion-molecule collision) has been reported for simple alcohols such as methanol[7] and ethanol[8] and, the phenomenon is also well-recognized for several other classes of compounds.[9] The electron-impact-induced fragmentation of the two tetrols is fully consistent with the structures (2) arrived at earlier; the fragmentation most relevant to the structure is summarised in 3 and 4 for the C_{18}-tetrol.

These tetrols have three asymmetric centres and hence for each tetrol four racemates are possible. The fact that in the plot of log of retention time *vs* chain-length, all compounds fall on a straight line, shows that, in all likelihood, the various long-chain aliphatic tetrols isolated from *Commiphora mukul* have the same configuration at the chiral centres. This is also supported by the fact that the two pure tetrols (C_{18}, C_{20}) display in their IR spectra identical absorption patterns in the C—O stretching region (1000–1200 cm^{-1}) and the PMR spectra of the derived pure acetonides are virtually superimposable in the δ 3·33–4·10



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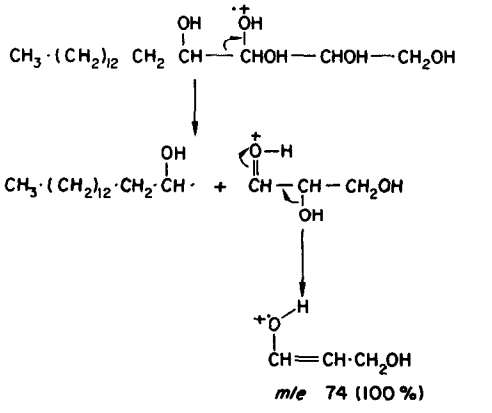

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region (protons on carbons linked to oxygen). In connection with the synthesis of phytosphingosines,[10] L-*arabino*-1,2,3,4-tetrahydroxyeicosane has been synthesised[11] and this compound has a m.p. (116–119°) quite distinct from that of the tetrahydroxyeicosane isolated from *Commiphora mukul*. Hence, our compound must have a configuration different from that of the synthetic product. On the other hand (±)-*xylo*- and (±)-*arabino*-1,2,3,4-nonadecanetetrol and (±)-*lyxo*- and (±)-*ribo*-1,2,3,4-octadecanetetrols representing all the four possible stereoisomers of long-chain 1,2,3,4-alkane-tetrols have been synthesised and their spectra recorded[12] and thus, it should be possible to deduce the configuration of the tetrols from *Commiphora mukul*. However, in practice, it has not been possible to draw any conclusion, since the published spectra are given for Nujol phase and comparison is not straightforward. We like to report on the absolute configuration of the compounds from *Commiphora mukul* in a future publication.

Sphingolipids are biologically important[13] and the biosynthesis of sphingosines is believed to involve serine and a long chain fatty acid coenzyme A.[10] Any link between the newly discussed alkane tetrols and phytosphingosine will be of obvious biosynthetic interest.

EXPERIMENTAL

All m.ps and b.ps are uncorrected. Light petroleum refers to the fraction b.p. 40–60°. Optical rotations were measured in EtOH on a Perkin-Elmer polarimeter, model 141.

SiO_2-gel for column chromatography was −100/+200 mesh and was washed with hot distilled water till sulphate-free, dried and activated at 125–130° (6–8 hr) and standardised.[14] TLC was carried out on SiO_2-gel layers (0·3 mm) containing 15% gypsum.

The following instruments were used for spectral/analytical data: Perkin-Elmer Infracord, model 137-E (IR); Varian Associates T-60 spectrometer (PMR; TMS as internal standard); CEC mass spectrometer, model 21-110B (Mass; 70 eV, direct inlet system); Hewlett-Packard-700 model (analytical GLC of acetonides; 180 cm × 0·5 cm Al column packed with 10% diethylene glycol succinate on Chromosorb W of 60–80 mesh, H_2 as carrier gas); 'Aerograph' model A-350-B (preparative GLC of acetonides; 300 cm × 1 cm Al column packed with 10% diethylene glycol succinate on Chromosorb W of 30–60 mesh, H_2 as carrier gas). While citing PMR data the following abbreviations have been used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and b (broad); the chemical shift position given is that of the centre of the signal. While summarising mass spectral data, besides the molecular ion, ten important ions (above *m/e* 50) are given with their relative intensities.

Long-chain aliphatic homologous tetrols (2)

The most polar gummy solid (10·55 g, eluted with 2–50% MeOH in EtOAc) from the chromatography[1] of the neutral material from the hydrolysis of the EtOAc extract of *guggulu*, was rechromatographed on SiO_2-gel/3b (550 g; 90 cm × 4 cm) using increasing amounts of EtOAc in C_6H_6 followed by MeOH in EtOAc. The major waxy solid (8·6 g), eluted with 2–10% MeOH in EtOAc, was recrystallised several times from Me_2CO to furnish colourless prismatic needles (3·0 g) m.p. 73–77°; TLC on 5% H_3BO_3-silica gel (solvent, 90% Me_2CO in light petroleum; double irrigation): 2 spots. Preparative chromatography (PLC, IDCC[15]) using the same adsorbent and solvent system, however, failed to achieve the analytical separation.

Tetra-acetate of 2. The tetrols (2, 10·0 g) in dry pyridine (30 ml) was acetylated with Ac_2O (30 ml) at 25° for 20 hr (TLC monitoring; 5% EtOAc in C_6H_6). The resulting glassy solid (14·4 g) was recrystallised several times from light petroleum to furnish snow-white silky needles (8·5 g), m.p. 42–43°.

Tetra-formate of 2. The tetrols (2, 0·20 g) was formylated with acetic-formic anhydride (5 ml) at 25° for 24 hr. The reaction mixture was taken to dryness at 27°/2 mm and the residue (TLC, 20% EtOAc in C_6H_6: 2 spots, different from starting tetrols) further formylated with more reagent (5 ml) for 24 hr. The crude solid (0·22 g, m.p. 66–68°), essentially single by TLC, was recrystallised several times from light petroleum to furnish white crystals of the tetra-formate (0·136 g), m.p. 68–70°.

Quantitative periodic acid oxidations

The tetrol (3) consumed 2·86 mole equivalent of H_5IO_6 according to a standard procedure[3] (25°, 1·5 hr). In a separate oxidation the liberated HCOOH was estimated by the HgO method,[4] when a value of 1·57 mole equivalent of HCOOH was obtained.

H_5IO_6-*Oxidation of tetrols* (2)

2,4-Dinitrophenylhydrazone of generated aldehyde. The tetrols 2 (0·634 g) was treated with a soln of H_5IO_6 (2·0 g) in water (80 ml) and AcOH (320 ml) at 25° and kept in the dark for 20 hr. Usual work-up gave a brownish liquid (0·573 g); TLC (solvent, 10% ether in light petroleum): one major spot and several minor. The major compound (0·373 g) was isolated by IDCC[15] (SiO_2-gel 125 g; 25 cm × 3·3 cm solvent: 10% ether in light petroleum) and distilled: colourless liquid, b.p. 120–140° (bath)/0·5 mm. This product was directly converted into its *2,4-dinitrophenyl hydrazone* (H_2SO_4-method) orange-yellow flakes from EtOH, m.p. 88–90°; TLC (solvent, 75% C_6H_6 in light petroleum): single.

Octadecane-1,2,3,4-tetrol

Bis-acetonide of 2. The tetrols (2, 0·385 g) in Me_2CO (20 ml) containing 60% $HClO_4$ (0·5 ml) was stirred at 25° for 16 hr, made alkaline (sat $NaHCO_3$ aq) and diluted with H_2O (50 ml). Acetone (~ 15 ml) was distilled off and the residue worked up in the usual manner to give a liquid (0·476 g); a small amount of base-sticking material (TLC: solvent, C_6H_6) was eliminated by filtration through neutral Al_2O_3 using light petroleum as solvent. The bis-acetonide was distilled: colourless liquid, b.p. 200° (bath)/0·02 mm; GLC (285°, flow rate 85 ml/min).

Bis-acetonide of octadecane-1,2,3,4-tetrol. The above acetonide mixture (~ 1 g) was separated by preparative GLC (300°, flow rate 90 ml/min, injection size: 20 μl) and the two major components RRT 1·65 (0·215 g) and RRT 2·71 (0·120 g) were isolated.

The acetonide with RRT 1·65 was distilled: colourless liquid b.p. ~ 200° (bath)/0·02 mm, m.p. 37–38°. Mass spectrum: important ions at *m/e* 398 (M^+, 6%), 383 (100%), 297 (89%), 59 (73%), 101 (53%), 55 (38%), 57 (32%), 384 (25%), 83 (22%), 69 (22%) and 97 (21%). (Found: C, 73·30; H, 11·88. $C_{24}H_{46}O_4$ requires: C, 72·31; H, 11·63%).

Octadecane-1,2,3,4-tetrol. The above pure acetonide (RRT 1·65; 50 mg) in dioxane (10 ml) was treated with 10% $HClO_4$ aq (1 ml) and stirred at 25° for 1 hr. The product was isolated with EtOAc as a sticky solid; TLC (solvent, EtOAc-MeOH-AcOH, 95:5:0·2): essentially single spot. Recrystallisation from EtOH furnished colourless needles (22 mg), m.p. 80–82°, $[\alpha]_D$ + 11·4° (*c* 0·34%). Mass spectrum: important ions at *m/e* 319 ($M+1^+$ 0·05%), 74 (100%), 56 (21%), 57 (20%), 55 (20%), 69 (14%), 85 (12%), 61 (10%), 73 (9%), 75 (8%) and 97 (7%). (Found: C, 69·04; H, 12·65. $C_{18}H_{38}O_4$ requires: C, 67·88; H, 12·03%).

Eicosane-1,2,3,4-tetrol

Bis-acetonide of eicosane-1,2,3,4-tetrol. The acetonide with RRT 2·71 (see above) was distilled: colourless liquid, b.p. ~ 200° (bath)/0·02 mm. Mass spectrum: important ions at *m/e* 426 (M^+ 1%), 59 (100%), 101 (64%), 325 (55%), 55 (48%), 411 (44%), 57 (36%), 69 (31%), 97 (29%), 83 (29%) and 95 (28%). (Found: C, 74·15; H, 12·39. $C_{26}H_{50}O_4$ requires: C, 73·19; H, 11·81%).

Eicosane-1,2,3,4-tetrol. Regeneration of the tetrol from the above acetonide (56 mg) was carried out as described earlier (see above). The crude tetrol was recrystallised from EtOH to furnish colourless needles (19 mg), m.p. 85–87°, $[\alpha]_D$ + 9·6° (*c* 0·37%). Mass spectrum: important ions at *m/e* 347 ($M+1^+$, 0·14%), 74 (100%), 57 (35%), 56 (35%), 55 (35%), 83 (29%), 73 (28%), 61 (25%), 75 (24%), 97 (20%) and 71 (19%). (Found: C, 70·78; H, 12·55. $C_{20}H_{42}O_4$ requires: C, 69·31; H, 12·22%).

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