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Isolation of new straight chain compounds from *Acacia nilotica*

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A novel diester, pentacosane dioic acid dihexadecyl ester **1** has been isolated for the first time from *n*-hexane soxhlet extract of *Acacia nilotica*. Saponification of the diester reveals the presence of an alcohol, hexadecanol and pentacosane dioic acid. The structures of both the compounds are confirmed by GCMS analysis. Saponification of pet-ether extract of *Acacia nilotica* has been carried out. Unsaponifiable matter is further purified by column chromatography followed by repeated mixed solvent crystallization to afford heptacosan-1,2,3-triol **2**.

Keywords: *Acacia nilotica*, diester, pentacosane dioic acid, saponification

IPC: Int.Cl.[8] A 61 K

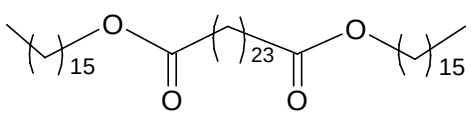
The diester **1** is a new compound. Literature survey revealed some esters with same molecular formula which are docosanoic acid-1,3-tridecanediyl ester, heneicosanoic acid-1,15-pentadecandiyl ester, heptadecanedioc acid-dieicosyl ester, nonadecanedioic acid-dinonadecyl ester, pentadecanedioc acid-diheneicosyl ester and tridecanedioc acid-didocosyl ester, etc[1].

Another compound **2**, heptacosan-1,2,3-triol is found to be new compound. Some diols/triols with same molecular formula are reported in the literature. They are 3-methoxy-4-methyl-9,11-pentacosane-diol[2], 2-hexadecyloxy-methyl-1,2-decane-diol[3] and 4-methyl-3,9,11-hexacosane-triol[4].

Results and Discussion

Compound **1**, a white solid, $C_{57}H_{112}O_4$, purified by repeated mixed solvent crystallization shows m.p. 63°C. In LCMS the molecular ion peak is not observed but a peak at m/z 412.5 indicates McLafferty rearrangement associated with hydrogen shift by two electron transfer due to symmetrical diester nature and the removal of a neutral molecule, 1-hexadecene, $C_{16}H_{32}$. The base peak at m/z 242.3 (radical ion 241 +1) is obtained by an ethereal oxygen fission from both sides of diester. IR spectrum shows sharp characteristic band at 1735.8 cm^{-1} (an ester carbonyl) and a peak at 1176.5 cm^{-1} (C-O ester ethereal oxygen). ^{1}H NMR spectrum shows multiplet at δ 4.06 for methylene protons attached to ester ethereal oxygen (4H, O=C-O-C*H*$_2$-), triplet at δ 2.29 for methylene protons attached to ester carbonyl (4H, -O=C-C*H*$_2$-, *J*= 5 Hz), multiplet at δ 1.61 for methylene protons (4H, -O-CH$_2$-C*H*$_2$-), a broad singlet at δ 1.26 for methylene proton envelops and a triplet at δ 0.89 (6H, *J*= 5 Hz) for two methyl groups. The ^{13}C NMR spectrum of compound **1** shows signals at δ 173.50 (*s*) for ester carbonyl group, 64.39 (*t*, O=C-O-*C*H$_2$-), 31.93 (*t*, CH$_3$-CH$_2$-*C*H$_2$-), 29.69 (*t*, -*C*H$_2$- envelop), 28.71 (*t*, O=C-O-CH$_2$-*C*H$_2$-), 25.94 (*t*, O=C-O-C*H*$_2$-CH$_2$-*C*H$_2$-), 22.71 (*t*, CH$_3$-*C*H$_2$-) and 14.11 (*q*, *C*H$_3$-CH$_2$-).

Saponification of compound **1** indicates the presence of pentacosane-dioic acid ($C_{25}H_{46}O_4$) and hexadecanol from GC-MS analysis. The data suggest that according to Wiley Dictionary there is 97%



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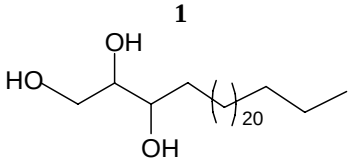

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probability of tricosane (after loss of $2CO_2$ from pentacosane-dioic acid) at 19.846 min and hexadecanol at 20.159 min. The data support the spectral data for confirming the structure of diester as pentacosane dioic acid dihexadecyl ester. This compound has been isolated for the first time.

Compound **2**, a straight chain triol, white solid, $C_{27}H_{56}O_3$, purified by repeated mixed solvent crystallization has m.p. 120°C. It shows molecular ion peak at m/z 427 (M^+-1) in negative mode of ionization of LCMS. IR spectrum shows a characteristic band at 3296.1 cm^{-1} (hydroxyl) and a peak at 1028.0 cm^{-1} (C-O stretching). ^{1}H NMR spectrum shows triplet at δ 3.65 for methylene protons overlapped with methyne protons attached to hydroxyl group (4H, -C*H*$_2$-OH and -C*H*-OH), a broad singlet at δ 2.04 for hydroxyl protons, multiplets at δ 1.69 and 1.61 for methylene protons (2H, -C*H*$_2$-CH-OH), a broad singlet at δ 1.26 for methylene proton envelop and a triplet at δ 0.89 (3H, *J*= 5 Hz) for methyl group. The ^{13}C NMR spectrum of compound **2** shows signals at δ 78.64 (*d*, OH-CH$_2$-*C*H-OH), 76.0 (*d*, OH-CH$_2$-CH-OH-*C*H-OH), 62.68 (*t*, OH-*C*H$_2$-), 32.55 (*t*, -*C*H$_2$-CH-OH), 31.61 (*t*, C*H*$_3$-CH$_2$-*C*H$_2$-), 29.14 (*t*, *C*H$_2$- envelop), 25.47 (*t*, -*C*H$_2$-CH$_2$-CH-OH), 22.36 (*t*, CH$_3$-*C*H$_2$-) and 13.77 (*q*, *C*H$_3$-CH$_2$-). This data indicate compound **2** to be heptacosan-1,2,3-triol. This compound has been found to be new after conducting literature survey.

Experimental Section

IR spectrum was recorded on a FTIR-8300 SHIMADZU spectrometer, NMR spectra on a Bruker DRX-500 operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR spectrum and a Bruker FT AC-200 instrument, operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C NMR spectrum at 24°C using residual signal of non-deuterated solvents as internal reference. Mass spectrum (EIMS) was recorded on Finnigan-Mat 1020 C mass spectrometer using ionization energy of 70 eV and LCMS on LCMS-MS Perkin-Elmer Applied Biosystems SCIEX- 2000. GC-MS analysis was done on Shimadzu QP 5050A mass spectrometer coupled to a Shimadzu 17A gas chromatograph fitted with a split-splitless injector.

Plant material—The aerial parts of *Acacia nilotica* (L.) Willd (Mimosaceae) were collected from forests near Pune. The plant specimen was authenticated by matching with the voucher specimen AHMA: 17000 available with the Agharkar Herbarium of Maharashtra Association, Pune, India.

Isolation of compound 1

Air shade dried, powdered plant material of *A. nilotica* (100.0 g) was extracted by continuous soxhlet extraction using solvents in the order: *n*-hexane, chloroform and ethanol. The *n*-hexane extract (D, 2.110 g) was used for further separation.

Chromatographic separation of constituents of extract D

The extract D (1.862 g) was fractionated over silica gel CC (1:30g) starting with *n*-hexane (800 mL) followed by *n*-hexane-ethyl acetate; 19:1 (3400 mL), *n*-hexane-ethyl acetate; 9:1 (200 mL), *n*-hexane-ethyl acetate; 17:3 (200 mL), *n*-hexane-ethyl acetate; 7:3 (200 mL), *n*-hexane-ethyl acetate; 1:1 (200 mL), *n*-hexane-ethyl acetate; 1:4 (200 mL) and finally with ethyl acetate. The fractions of 200 mL volume were collected. The progress of the column chromatographic separation was monitored by performing thin layer chromatography of the fractions. Fractions showing similar compositions were combined together to obtain eighteen major fractions (1-18).

Rechromatography of fraction 3

Fraction 3 (0.612g), containing compound **1** was rechromatographed by column chromatography on silica gel (1:70g) starting with *n*-hexane (50 mL) followed by *n*-hexane-toluene; 1:1 (100 mL), *n*-hexane-toluene; 1:4 (200 mL), toluene (100 mL), toluene-ethyl acetate; 24:1 (100 mL), toluene-ethyl acetate; 9:1 (100 mL), toluene-ethyl acetate; 1:1 (100 mL) and finally ethyl acetate (50 mL). The fractions of 50 mL volume were collected. The progress of the column chromatographic separation was monitored by thin layer chromatography of the fractions. Fractions showing similar compositions were combined together to obtain eight major fractions (1-8). The column chromatography afforded impure compound **1** (0.187g). It was purified by repeated crystallization using chloroform-methanol to give pure compound **1** (0.079 g).

To confirm the diester nature of the compound **1**, it was saponified. A solution of potassium hydroxide (2%, MeOH-H_2O; 9:1, 10 mL) was added to the compound **1** (20 mg). The mixture was refluxed for 4hr. Usual work-up procedure yielded an alcohol (15 mg) and an acid (5 mg). GC-MS analysis of both the compounds was carried out.

Isolation of compound 2

Air shade dried, powdered plant material of *A. nilotica* (50.0 g) was extracted by refluxing with pet-

ether (60-80 fraction). The pet-ether extract was filtered, dried (E, 1.009 g) and saponified with KOH (10% in EtOH-H_2O; 50:50) by refluxing for 4hr. The alkaline solution was extracted with diethyl ether to separate acidic part from pet-ether extract. The ether layer was successively washed with distilled water to make neutral and dried over anhydrous sodium sulphate. Removal of ether furnished unsaponifiable matter, the extract F (1.00 g).

Chromatographic separation of constituents of extract F

The extract F (1.00 g), containing compound **2** was fractionated over silica gel CC (1:10 g) starting with pet-ether (300 mL) followed by pet-ether-ethyl acetate; 19:1 (400 mL), pet-ether-ethyl acetate; 9:1 (200 mL), pet-ether-ethyl acetate; 17:3 (200 mL), pet-ether-ethyl acetate; 3:1 (300 mL), pet-ether-ethyl acetate; 3:2 (200 mL) and finally with ethyl acetate (100 mL). The fractions of 100 mL volume were collected. The progress of the column chromatographic separation was monitored by performing thin layer chromatography of the fractions. Fractions showing similar compositions were combined together to obtain eight major fractions (1-8). The column chromatography afforded impure compound **2** (0.16g).

Compound **2** was purified by passing through sephadex LH 20 column and by repeated crystallization using pet-ether to yield fatty needles of compound **2** (0.102 g).

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