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CHEMICAL CONSTITUENTS *OF SAPINDUS MUKOROSSI* GAERTN. (SAPINDACEAE)

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ABSTRACT

Six new fatty esters of tetracyclic triterpenoid (**1-6**) together with a known acyclic sesquiterpenoidal glycoside (**7**) have been isolated from the fruits of *Sapindus mukorossi* belonging to the family Sapindaceac. Their structures were elucidated with the help of extensive spectroscopic techniques and by chemical means.

Introduction

During our phytochemical investigations on terrestrial plants, we examined *Sapindus mukorossi (Perry*, 1980) belonging to the family Sapindaceac having 150 genera and 2000 species. The trees are cultivated in many parts of India for ornamental purposes. The chemical literature survey revealed that the major emphasis was given to its fruits due to their expectroant and emetic property and also for the presence of saponins (Gedeon, 1954, Yasukhiko and Shimidhi, 1961, Garg *et al*, 1982). The saponins from this plant are used as a level-dyeing agent in dye baths, as an emulsifier in the preparation of insecticides, faom stabilizer in the manufacture of soapless shampoos, germicides and deodorizing agents. A number of chemical constituents (Zikova *et al*, 1973) belonging to the classes saponins (Kitasato and Sono, 1932, Nakayama *et al*, 1986), flavonoids (Zikova, 1966), fatty acid esters (Sengupta *et at*, 1975) have been isolated from various species of the genus *Sapindus.*

Results and Discussion

Compounds 1-6 were obtained by repeated column chromatography of the fractions eluted with hexane:chloroform (9:1) from the silica gel column loaded by hexane extract. On the basis of spectral evidences these were assigned as a mixture of tetracyclic

triterpene esters and were found to he unseparatable due to their very dose R_f values. Alkaline hydrolysis of the mixture yielded a single triterpene moiety close R_f values. Alkaline hydrolysis of the mixture yielded a single triterpene moiety and a mixture of fatty acids which were first esterified with diazomethane, then identified by GC-MS as tetradecanoate, pentadecanoate, hexadecanoate, heptadecanoate, nonadecanoatc and heneicosanoatc. The high resolution mass spectrometry (HRMS) of the mixture showed the molecular ion peaks for all the esters at m/z 748, 720, 692, 678, 4 and 650 corresponding to the molecular formulae $C_{52}H_{92}O_2$, $C_{50}H_{88}O_2$, $C_{45}H_{84}O_2$, $C_{47}H_{82}O_2$, $C_{46}H_{80}O_2$ and $C_{45}H_{78}O_2$ respectively. The IR spectrum of the mixture showed strong absorption band for the ester carbonyl function at 1735 cm^1.

The field descorption mass spectrum of compounds 1-6 showed the molecular ion peaks at m/z 650, 664, 678, 692, 720 and 748. The electron impact mass spectrum (El-MS) also showed these peaks and the other intense fragments at m/-z 410, 393, 313, 259, 229, 205 and 153. The fragmentation pattern showed the presence of 7, 24 or 9(11), 24 euphane tirucallane series (m/z 231, 243 and 257 for 7 or 9(11)-enc, m/z 297 for tetracyclic triterpenoid, and m/z 69 for 24 ene) (Wyllie and Djerrasi, 1968, Goad et *al.*, 1967). The ^{1}H-NMR spectrum of the mixture showed the presence of five tertiary methyl signals which must be localized in the steroid skeleton and appeared as singlets at δ 0.76, 0.80, 0.85, 0.93 and 0.97. The chemical shifts of these methyl singlets were very close to eupha-7, 24 dienol (Teresa *et al*, 1987). A triplet at δ 0.87 (J = 6.7 HZ) due to a primary methyl was attributed to a terminal methyl of the fatty acids. The ^{1}HNMR spectrum further showed two olefinic proton signals at δ 5.09 and 5.23, out of which one (δ 5.09) must he on the isopropylidene moiety of the side chain, because the vinyl methyl signals (26, 27-methyls) were present in the spectrum at δ 1.59 and 1.67 (Iloh *et al*, 1976). The intensity of an ion at m/z 69 [100%, $(C_5H_9)^+$] in the mass spectrum, which arose from allylic cleavage of the C(22) - C(23) bond, strongly sup-ported the presence of the double bond at C-24 and the presence of isopropylidine group in the side chain. The presence of an axial proton at C-3 which appeared as double doublet at 4.50 (J=11.0, 4.0 Hz) indicated the 3β-configuration of the ester group (Bhacca and Williams, 1964). Therefore the triterpene moiety in the compounds **1-6** was found to be eupha 7, 24-diene-3β-o1.

The carbon nuclear magnetic resonance spectrum of the mixture further showed the conclusive evidences to support the proposed structures. It showed the signals due to eight methyls of aglycone moiety at 3 13.71, 15.96, 17.67, 18.60, 22.08, 25.70, 27.35 and 27.64. Another methyl group signal at S 14.09 was assigned to terminal methyl of fatty acid chains. The downfield shifting of signal of C-3 at b 80.85 showed the attachment of fatty acids at this position. The ^{13}C-NMR spectrum also showed two sets of trisubstituted olifinic carbons at 6 117.65, 146.04 and 125.18, 130.89, characteristics of Δ^7 and Δ^{24} cuphane. Further, the spectrum showed the presence of ester carbonyl group resonance at

6 173.62, a terminal methyl group of fatty acid at 6 1.4.09, and a bunch of carbon signal at 6 29.2-29.7 assigned to $(CH_2)_n$ of fatty acid chain.

On the basis of all the above evidences the structures of compounds 1-6 were assigned as eupha, 7, 24-dicne-3-tetradecanoale, eupha 7, 24-dien-3-penladecanoate, eupha 7, 24-dien-3-hexadecanoate cupha 7, 24-dien-3-heptadecanoate, eupha 7, 24 dien-3-nonadccanoatc and cupha 7, 24-dicne-hcneicosanoate, respectively.

Structure Elucidation of Compound (7)

The crude fractions obtained with chloroform: methanol (3:1) cluate from the first silica gel column furnished compound **7** with some minor impurities. It was further purified by HPLC on a reverse phase (RP-18) column. The elution was carried out with methanol: water (3:1). The compound 7 showed strong absorption hands in the hydroxyl region of the IR spectrum indicating the glycosidie nature. The ^{1}H-NMR spectrum showed the presence of six anomcric proton resonance at δ 4.35 (d, J=7.6 Hz), 4.38 (d, J=7.7 114 490 (d, J=1.46 Hz), 4.91 (d, J=1.43 Hz), 4.95 (d, J =1.45 Hz) and 4.96 (d, J=1.46 Hz) indicating the presence of six sugar moieties. It further showed the presence of three olefinic proton resonances each linked to a methylene group and appeared as triplet at d 5.14 (J=6.6 Hz), 5.38 (J=6 2 Hz) and 5.46 (J=6.8 Hz). The ^{1}H-NMR spectrum also showed the presence of three vinyl methyl singlets at δ 1.69, 1.68 and 1.62.

Acid hydrolysis of 7 yielded rhamnose and glucose which were identified by comparing with authentic samples on silica gel TLC. The ^{13}C-NMR spectrum showed teen signals due to aglycone moiety and thirty six signals due to six sugar units. The three methyl signals appeared at δ 14.75, 16.19 and 16.58. The olefinic carbon resonances occured at δ 121.47, 125.39, 130.68, 132,71, 136.13 and 142.02. Comparison of the signals due to aglycone with those of geraniol, all *trans* farnesol and other related compounds formulated the aglycone of compound **7** as 12-hydroxy-all-*trans*-farnesol (Wehrli and Nishida, 1979, Tschesche *et al.,* 1977). Moreover, the ^{13}C-NMR spectrum showed six anomcric carbon signals at δ 101.07, 101.38, 102.78, 103.06, 103.79 and 103.80. Study of the carbon signals due to sugar moieties exhibited the presence of four terminal rhamnose and two inner glucose (Gorin and Mazurek, 1975). The downfield signals of C-2 and C-3 of both glucose units indicated the attachment of all the four rhamnose moieties at these carbons. Comparison of the ^{1}H- and ^{13}C-NMR data with known related compounds indicated that the sugar moieties attached to the 1- and 12-hydroxy positions of aglycone were identical, that is rhamnose (1→2) glucose (3→1) rhamnose (Kasai *et al,* 1986, Sakamoto *et al,* 1977, Kasai *et al*., 1979).

Sequences of the sugar moieties were also confirmed by FAB mass spectrometry

which showed the $[M-H]^-$ peak at m/z 1045 corresponding to the molecular formula $C_{15}H_{68}O_{28}$. The other intense fragments at m/z 999, 853, 691, 545 and 237 corresponding to the losses of rhamnose, 2 x rhamnose, 2 x rhamnosc + glucose, 3 x rhamnose + glucose and 4 x rhamnose + 2 x glucose moieties from $[M-H]^-$, respectively.

All the above mentioned evidence have led to conclude that the structure of compound **7** as 1, 12-bis-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl (3→1)-α-L-rhamnopyranosyl all-*trans* farnesol.

Experimental

The nuclear magnetic resonance spectra were recorded on Bruker AM-4M and 1(X) for ^1H and ^{13}C-nuclei, respectively.

Fruit's shells of *Sapindus mukorossi* (Ritha, 6 kg) were separated from seeds, then crushed (3 kg) in homogenises and extracted three times in hexane (about 10 lit.) The combined hexane extract was evaporated under reduced pressure. The crude hexane residue (25 g) was subjected to column chromatography using solvent systems hexane, hexane : chlorform, chloroform, chloroform : methanol and finally *pure* methanol. The fractions eluted with 9:1 (hexane: chloroform) yielded compounds **1-6**.

Compound **7** was obtained by HPLC of the crude fractions eluted with chloroform : methanol (3:1) from the silica gel column. A reverse phase semi-preparative (RP-18) column was used for purification and methanol : water (3:1) was used as mobile phase.

Characterization of Compounds 1-6

Repeated column chromatography of the fractions eluted with hexane : chloroform (9:1) yielded a mixture of letracyclic triterpenoidal esters (**1-6**). Alkaline hydrolysis of the mixture gave a single tetracydic triterpene and a mixture of fatty acids, identified by GC-MS after methylation with diazomethane.

EI-MS m/z (rel.int %): 748 (4), 720 (2), 692 (2), 678 (4), 664 (10), 650 (30), 410 (10), 408 (6), 393 (80), 313 (I), 311 (2), 297 (3), 259 (4), 229 (15), 218 (16), 205 (20). 203 (12), 190 (20), 173 (16), 153 (20), 123 (24), 109 (54), 95 (50), 81 (34), 69 (100). 55 (54).

HR-MS m/z : 748.7197 (M^+, $C_{52}H_{92}O_2$, calcd. 748.7897), 720.6844 (M^+, $C_{50}H_{88}O_2$, calcd. 720.6784), 692.6435 (M^+, $C_{48}H_{84}O_2$, calcd. 692.6471), 678.6199 (M^+, $C_{47}H_{82}O_2$, calcd. 678.6315), 664.6133 (M^+, $C_{46}H_{80}O_2$, calcd. 664.6158), 650.5937 (M^+ $C_{45}H_{78}O_2$,

calcd. 650.600), 410.3851 (M-fatly acid)$^+$, 408.3755 (M-fatty acid-2H)$^+$, 393.350 (M-fatty acid-2H-Me)$^+$.

Alkaline Hydrolysis of Compounds 1-6

Compounds 1-6 (10 mg) were refluxed with 2% sodium hydroxide in a mixture of methanol : water (7:3) for 2 hours. The reaction mixture was cooled at room temperature and extracted with chloroform to obtain the triterpene. The aqueous layer was separated, neutralized with dilute hydrochloric acid and extracted with chloroform. The chloroform *layer* was evaporated and the residue so obtained was analysed by GC-MS after methylation with diazomethanc to determine the fatty acid composition.

^{1}H-NMR ($CDCl_3$, 400 MHz) : δ 0.76 (s, CH_3), 0.80 (s, CH_3), 0.85 (s, CH_3), 0.85 (d, J =6.30 Hz, CH_3), 0.93 (s, CH_3), 0.97 (s, CH_3), 1.59 (d, J = 0.60 Hz. CH_3). 1.67 (d, J=1(14 Hz, CH_3), 3.32 (dd, J=11.00, 4.00 Hz, H-3), 5.09 (m, H-24) and 5.23 (dd, J=6.70, 2.90 Hz, H-7).

^{13}C-NMR ($CDCl_3$, 100.01 MHz) : δ 36.90 (C-1), 24.30 (C-2), 80.85 (C-3), 37.95 (C-4), 50.87 (C-5), 23.83 (C-6), 117.65 (C-7), 146.04 (C-8), 48.91 (C-9), 34.89 (C-10), 18.21 (C-11), 33.84 (C-12), 43.61 (C-13), 51.34 (C-14), 34.02 (C-15), 28.46 (C-16), 53.30 (C-17), 13.71 (C-18), 22.08 (C-19), 35.81 (C-20), 18.60 (C-21), 35.26 (C-22), 25.41 (C-23), 125.18 (C-24), 130.89 (C-25), 17.67 (C-26), 25.70 (C-27), 27.64 (C-28), 27.35 (C-29) and 15.96 (C-30).

Fatty acid (methyl esters)

GC-MS, m/z (rel.int. %):

Tetradecanoate : 242 (M^+, $C_{15}H_{30}O_2$) (18), 185 (M^+ -57) (10), 157 (5), 143 (15), 129 (10), 87 (54), 74 (100), 69 (14) and 55 (24).

Pentadecanoate : 256 (M^+, $C_{16}H_{32}O_2$) (74), 213 (M^+-43) (27), 199 (4), 185 (14), 171 (12), 157 (12), 143 (7), 129 (35), 115 (10), 101 (8), 87 (25), 73 (90) and 59 (100).

Hexadecanoate : 270 (M^+, $C_{17}H_{34}O_2$) (30), 239 (M^+-39) (10), 227 (M^+-43) (12), 213 (2), 199 (5), 185 (7), 171 (4), 157 (6), 143 (14), 129 (6), 115 (22), 101 (5), 87 (60) and 73 (100).

Heptadecanoate : 284 (M^+, $C_{18}H_{36}O_2$) (35), 241 (M^+-43) (16), 213 (5), 199 (7), 185 (6), 171 (2), 157 (14), 143 (8), 129 (4), 115 (6), 101 (55), 88 (100), 73 (17) and 59 (25).

Nonadecanoate: 312 (M^+, $C_{20}H_{46}O_2$) (35), 281 (M^+-31) (2), 269 (M^+-43) (14), 255 (3), 141 (2), 227 (2), 213 (8), 199 (2), 171 (3), 157 (16), 143 (8), 129 (5), 115 (8), 101 (55), 88 (100) and 57 (40).

Heneicosanoate : 340 (M^+, $C_{22}H_{44}O_2$) (15), 297 (M^+-43) (2), 283 (25), 267 (18), 241 (5), 227 (2), 199 (2),185 (10), 171 (5), 141 (3), 129 (14), 115 (10), 101 (8), 73 (25) and 56 (1.00).

Characterization of Compound 7

1,12-his-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(3→l)-α-L-rhamnopyranosyl all-*trans* farnesol.

^{1}H-NMR (CD_3OD, 300 MHz): δ 1.20 (d, J=648, 2xCH_3, H-6, rha'^{a}, rha"^{a}), 1.25 (d, J =6.16 Hz, 2xCH_3, H-6, rha$^{'''a}$, rha$^{''''a}$), 1.62 (s, CH_3), 1.68 (s, CH_3), 1.69 (s, CH_3), 1.97-2.16 (m, 4xCH_2), 4.35 (d, J =7.6 Hz, H-1, gl^b), 4.38 (d. J=77 Hz, H-1, g2^b), 4.90 (d, J=1.46 Hz, H-1, rha$^{'c}$), 4.91 (d, J=1.47 Hz, rha$^{''c}$), 4.95 (d, J=1.45 Hz, H-1, rha$^{'''c}$), 4.96 (d, J=1.46Hz, H-1, rha$^{''''c}$), 5.14 (t, J=6.6 Hz, H-6), 538 (t, J=6.6 Hz, H-2) and 5.46 (t, J= 6.8 Hz, H-10).

a,b,c: assignments may be interchanged.

^{13}C-NMR (CD_3OD, 75.43 MHz) (Aglycone): δ 66.45 (C-1), 121.47 (C-2), 142.02 (C-3), 40.33 (C-4), 27.38 (C-5), 125.39 (C-6), 136.13 (C-7), 40.65 (C-8), 27.53 (C-9), 130.68 (C-10), 132.71 (C-11), 76.11 (C-12), 16.58 (C-13), 16.19 (C-14) and 14.75 (C-15).

Sugar moieties

Glucose (g_1)

δ 101.07 (C-1)^a, 80.19 (C-2)^b, 88.20 (C-3)^c, 70.23 (C-4)^d, 77.61 (C-5)^e, 62.68 (C-6)^f.

Glucose (g_2)

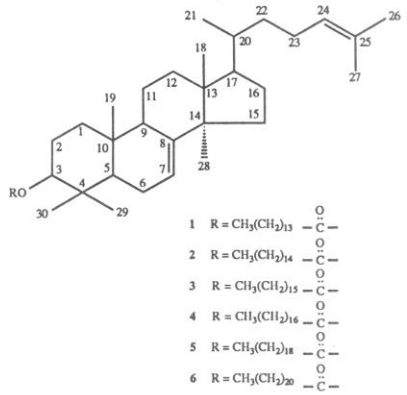
δ 101.38 (C-1)^a, 79.45 (C-2)^b, 88.45 (C-3)^c, 70.23 (C-4)^d, 77.68 (C-5)^e. 62.68 (C-6)^f.

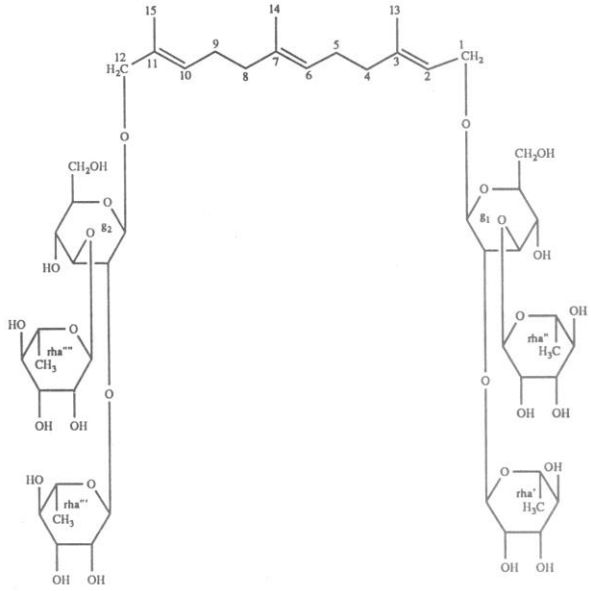
Rhamnose'

δ 102.78 (C-1')^a, 72.29 (C-2), 72.34 (C-3)^b 73.66 (C-4)^c, 70.63 (C-5)^d, 17.85 (C-6)^e.

Rhamnose"

δ 103.06 (C-1")[a], 72.29 (C-2), 72.50 (C-3)[b], 73.66 (C-4)[c], 70.70 (C-5)[d], 17.85 (C-6)[e].





Rhamnose"'

δ 103.79 (C-1"')[a], 72.29 (C-2), 72.50 (C-3)[b], 73.84 (C-4)[c], 70.86, (C-5)[d], 18.30 (C-6)[e].

Rhamnose""

δ 103.80 (C-1"")[a], 72.29 (C-2), 72.50 (C-3)[b], 73.84 (C-4)[c], 70.71 (C-5)[d], 18.03 (C-6)[e].

a, b, c, d,e ,f: assignments may be interchanged.

FAB-MS (negative mode, high field) m/z : 1145 $(M-H)^-$, 999 $(M-H-rha)^-$, 853 $(M-H-2 \times rha)^-$, 691 $(M-H-2 \times rha-glc)^-$, 707 $(M-H-3 \times rha)^-$, 545 $(M-H-3 \times rha-glc)^-$, 396 $(M-H-4 \times rha-glc)^-$ and 237 (M-4 x rha - 2xglc).

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