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# A NEW DIOSPYROL GLYCOSIDE FROM *DIOSPYROS MOLLIS* GRIFF.

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Abstract : A new compound from the leaves of *Diospyros mollis* Griff. was elucidated as diospyrol 8,8'-di-O-(6-β-D-apiofuranosyl-β-D glucopyranoside) by spectroscopic methods.

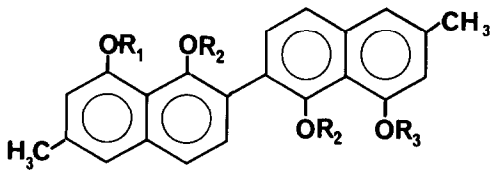
The berries of *Diospyros mollis* Griff. (Ebenaceae) are one of the oldest herbal drugs known in South East Asia countries for their anthelmintic properties (1). The active principle is a dinaphtol : diospyrol (2)(structure I). Recently an unstable diglycoside (structure III) from the green berries was isolated and then purified under its acetylated form (3). We report here the results of the chemical studies we have performed on the leaves of *Diospyros mollis* Griff. (4) which are easier to get than berries.

A crude extract of glycosides was obtained after ethanolic extraction of dried powdered leaves and organic solvents fractionation. The purification of the glycoside extract was not performed after a prior acetylation as mentionned in (3),but according to an original method: reversed phase ($C_{18}$) preparative high performance liquid chromatography (solvent : $CH_3OH/H_2O$; 3/2, v/v). We obtained an amorphous slightly brown product : compound V (0.5 % from dried leaves), with molecular formula $C_{44}H_{54}O_{22}$, 4 $H_2O$ (calculated from C 53.18 H 6.15 O 40.63), m.p. 190° (decomp.), U.V. spectra : $\lambda^{EtOH}_{max}$ nm (log ε) 226 (4.69) 240 (4.68) 260 (4.60) and 300-340 (4.17).

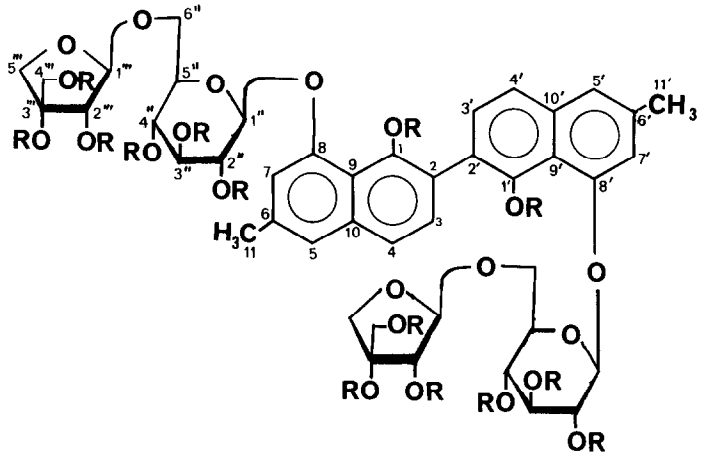
The acid hydrolysis of V carried out in refluxing HCl 2N for ∿ 7 mn afforded an instable aglycone, I, and two different sugars, identified by TLC and GLC as glucose and apiose (5).

The electron impact mass spectrometry of I showed a molecular ion at m/z = 346 for $C_{22}H_{18}O_4$ and a characteristic ion at m/z = 173 corresponding to the fragmentation of the dimeric compound. The U.V. spectra of methylated derivative of I is identical to the methylated diospyrol (6).

Compound V was acetylated ($Ac_2O/C_5H_5N$) into VI, m.p. = 130°. The $^1H$ NMR of VI (see TABLE 1) confirmed the diospyrol structure for the aglycone part. We could note the absence of both $C_8$ and $C_8'$ phenolic acetate at δ = 2.31 and the presence of phenolic acetate in $C_1$ and $C_1'$ at δ = 1.93, which implicated a glycosylation site in $C_8$ and $C_8'$. The 12 remaining acetate groups indicated the linkage of 2 molecules of hexose and 2 molecules of pentose.



I : $R_1 = R_2 = R_3 = H$
II : $R_1$ = (Glucose) $Ac_4$, $R_2 = R_3 = Ac$
III : $R_1 = R_3$ = Glucose, $R_2 = H$
IV : $R_1 = R_3$ = (Glucose) $Ac_4$, $R_2 = Ac$



V : R = H
VI : R = Ac

TABLE 1 - $^1H$ NMR of Compounds II[a], IV[a] and VI[b]
($CDCl_3$ solution, δ ppm with TMS as internal reference)

| position of proton (signal multiplicity)[c] | II | IV | VI |
|---|---|---|---|
| 1 and 1' acetate (s) | 1.93 | 1.92 | 1.93 |
| 3 and 3' (d) | 7.33[e] | 7.36[e] | 7.35[e] |
| 4 and 4' (d) | 7.65[e], 7.74[e] | 7.68[e] | 7.65e |
| 5 and 5' (d) | 7.39[f], 7.58[b] | 7.41[f] | 7.40f |
| 7 and 7' (d) | 7.02[f], 7.01[f] | 6.98[f] | 6.95[f] |
| 8 or 8' acetate (s) | 2.30 | - | - |
| sugar acetate (s) | 1.93-2.06 | 1.95-2.07 | 1.94, 1.97, 1.98 1.99, 2.05, 2.06 |
| others (m) | 3.70-5.10 | 3.70-5.10 | 3.50-5.40 |
| 11 and 11' (s) | 2.47 | 2.47 | 2.49 |

a - data from reference 3
b - CAMECA spectrometer at 350 MHz ; spectral width : 3521 Hz ; pulse flipping angle : 27°, acquisition time : 2.3 sec.
c - s = singlet, d = doublet, m = multiplet
e - ortho coupling J = 9 Hz
f - meta coupling J = 1.5 Hz

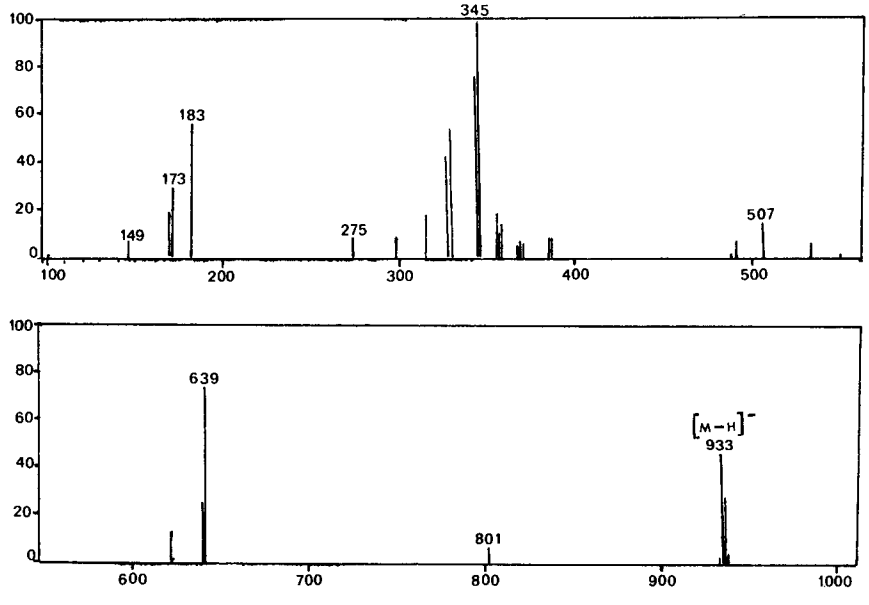
The negative Fast Atom Bombardement mass spectrometry (7) of V showed an important molecular proton abstracted ion at $(M-H)^-$ = 933 (46 %) (see Figure) confirming the $C_{44}H_{54}O_{22}$ molecular formula. The only important fragmentation mechanism involved successive expulsions of sugar molecules. At m/z = 801 (6 %) we found the ion corresponding to the loss of a terminal apiose (in the case of a terminal glucose, we would have found an ion at m/z = 771). The two other important ions on the spectrum were at m/z = 345 (100 %), corresponding to diospyrol, and m/z = 633 (74 %) showing the elimination of both one apiose and one glucose. We could also observe at m/z = 507 (15 %) the ion induced by the loss of two molecules of apiose and one of glucose.

We tentatively assigned $^{13}C$ NMR signals of V (see TABLE 2) by comparison with the data on $^{13}C$ chemical shifts of glucosides, apiosides (8) and substitued naphtalene (9), and by the $^1H$ single-frequency off resonance decoupling technique. The anomeric carbons appeared at $\delta$ = 110.6 (DMSO), 111.0 ($C_5D_5N$) and $\delta$ = 103.9 (DMSO), 104.5 ($C_5D_5N$) corresponding to a β-D-apiofuranoside and a β-D-glucopyranoside respectively. The linkage site between the two sugars could easily be determined by the observation of the downfield shift ($\sim$ + 6 ppm) of $C_6$" of the glucose moiety. These data confirmed the presence of two symetrical sugar units.

We can propose the structure V for this new compound.

A 6-β-D apiofuranosyl-β-D-glucopyranoside had been reported previously, with esculetin as aglycone, in another species : *Diospyros sapota* Roxb. (8b).

It is interesting to note that the diglucoside of diospyrol reported in literature (3) is extracted from the berries by a mixture of acetone/HCl (25/1 ; v/v) whereas the apiose moieties of V are very labile under acidic conditions. So in further investigations we will apply our soft technique of isolation to berries.




Negative FAB Mass spectra of compound V.

TABLE 2 : $^{13}C$ Chemical shifts of Compound V

($\delta$ ppm with TMS as internal reference)

| carbon | | solvent DMSO-$d_6$ [a] | solvent $C_5H_5N$-$d_5$ | c | carbon | | solvent DMSO-$d_6$ [a] | solvent $C_5H_5N$-$d_5$ [b] | c |
|---|---|---|---|---|---|---|---|---|---|
| diospyrol | 1 | 155.4 | 155.7 | s | glucose | 1" | 103.9 | 104.5 | d |
| | 2 | 121.2 | 121.2 | s | | 2" | 74.8 | 74.5 | d |
| | 3 | 132.2 | 131.8 | d | | 3" | 77.6 | 78.4 | d |
| | 4 | 123.3[e] | 122.5[e] | d | | 4" | 71.6 | 71.5 | d |
| | 5 | 118.7[e] | 117.6[e] | d | | 5" | 77.6 | 77.79 | d |
| | 6 | 136.9 | 136.8[f] | s | | 6" | 69 | 68.7 | t |
| | 7 | 114 | 133.4 | d | apiose | 1"' | 110.6 | 111 | d |
| | 8 | 151.5 | 152 | s | | 2"' | 77.6 | 77.5 | d |
| | 9 | 136.9 | 136.1[f] | s | | 3"' | 80.2 | 80.1 | s |
| | 10 | 114.7 | 114.9 | s | | 4"' | 64.7 | 65.5 | t |
| | 11 | 22.9 | 21.8 | q | | 5"' | 71.6 | 74.9 | t |

a - VARIAN XL-100 spectrometer at 25.2 MHz ; special width : 5500 ; pulse flipping angle : 35° ; acquisition time : 0.8 sec.
b - CAMECA 350 spectrometer at 88 MHz ; spectral width : 19230 ; pulse flipping angle : 38° ; acquisition time : 0.425 sec.
c - s = singlet, d = doublet, m = multiplet
e, f, g - Assignments may be interchanged in each vertical column.

## References and Notes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(4) The plant material, collected from Khorat Plateau (THAILAND) in August 1980, was identified in our laboratory.
(5) D-apiose was prepared from *Petroselinum sativum* Hoffm. (Parsley) according to the literature procedure : R.B. DUFF, Biochem. J. (1965) 94, 768 and D.J. BELL. Methods in Carbohydrate Chemistry (1962). Academic Press, New-York, 1, 260.
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(7) The spectrum was recorded using a VG Analytical ZAB-HF at accelerating potential of 8 KeV. The primary atom beam comprised Xe and was produced using a saddle field ion source (Ion Tech Ltd, B 11 N) operating with a tube current of 1 mA at on energy of 8 KeV. Sample of compound V was prepared in glycerol solution and deposited on a stainless steel sample support. (At m/z = 183 and 275 we have the ions of the glycerol matrix).
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