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A NEW DIGLUCOSIDE FROM THE ANTHELMINTIC BERRIES OF DIOSPYROS MOLLIS

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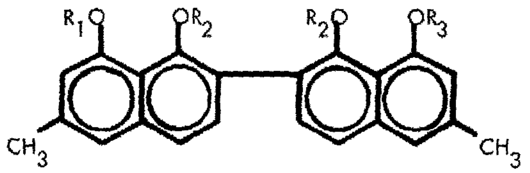
Chemical studies of the fresh berry of Diospyros mollis have been motivated by its anthelmintic efficacy and problems associated with its use in mass chemotherapy programs. Because the fruit is available for only a few months yearly and deteriorates rapidly soon after harvest, chemical knowledge is indispensable in solving problems related to isolation and preservation of active principles. The most critical, but still missing, piece of information is the complete identification of all potentially active compounds present Compounds identified by various investigators are · diospyrol (I) [1], a few oxidised products of diospyrol [2,3,4], and its putative monomeric precursor [5]. Only diospyrol has been isolated in large amount from the fresh berry by conventional solvent extraction procedures and has been widely assumed to be the active ingredient. In a mass chemotherapy program that we are involved recently, we noted that the effective dose of pure diospyrol is much higher than would be present in equipotent quantity of fresh berry. The search for other active components in fresh berry resulted in an extraction procedure that yields a new diglucoside of diospyrol as the main compound in lieu of diospyrol. We report here the detail of this discovery together with evidences which suggest that free diospyrol is not the main constituent of the fresh berry.

Fresh, green berries, harvested a few days before, were ground in acetone (2 kg in 2 L), the mixture was filtered and solvent discarded. The paste was extracted immediately with acetone at room temperature under nitrogen atmosphere. Concentration of the filtered acetone extract gave white precipitate of pure diospyrol (25 g, m.p. 205-215°decomp.) like procedures employing other solvents [1-4]. If the berries were ground in acetone containing HCl conc (25 1) and the rest of the procedure followed exactly, a pure white solid which is not diospyrol was obtained (4 g, m.p 180-210°decomp) Using TLC with several developing solvent systems and separation of acetylated derivatives, we have found that the unknown transforms into diospyrol spontaneously. Separation of acetylated products of the unknown (acetic anhydride/pyridine) on silica column afforded diospyrol tetraacetate (II) (5 %) and two other compounds. A pale yellow prismatic crystal (45 %, m p. 145°, m/e 802) was assigned as structure III Existence of structural unsymmetry due to the presence of only one C-8 sugar was evident from the n m.r. data. The last compound, identified as IV, was a pale yellow amorphous solid (7 %, m.p. 153 °). Molecular ion of this compound could not be obtained due to its high molecular weight, but its fragmentation ions and other spectroscopic properties are consistent with the assigned structure The presence of two symmetrical sugar units and the absence of both C-8 and C-8' phenolic acetate in a diospyrol structure are evident from the n.m.r data



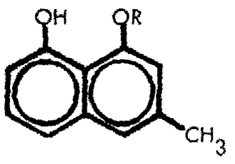
I diospyrol, $R_1 = R_2 = R_3 = H$

II $R_1 = R_2 = R_3 = Ac$

III $R_1 =$ glucose(Ac)$_4$, $R_2 = R_3 = Ac$

IV $R_1 = R_3 =$ glucose(Ac)$_4$, $R_2 = Ac$

V $R_1 = R_3 =$ glucose, $R_2 = H$



VI R = H

VII R = glucose

The conclusion that the sugar moieties are glucose was reached after comparing the sugars (and their derivatives)isolated from acid-hydrolysates of compounds III and IV with authentic ones and only glucose was found.

We conclude that the unknown isolated by the acid-acetone procedure was the diglucoside of diospyrol (V). Although we cannot yet explain why the condition allowed its isolation, we do not think it could be formed from pre-existing diospyrol. Our results therefore suggest that fresh, green berries contain precursors of diospyrol rather than the free form.

N.M.R. absorption (δ) of compounds II, III and IV (CDCl$_3$ solution, TMS as internal standard)

s= singlet, d= dublet, m= multiplet

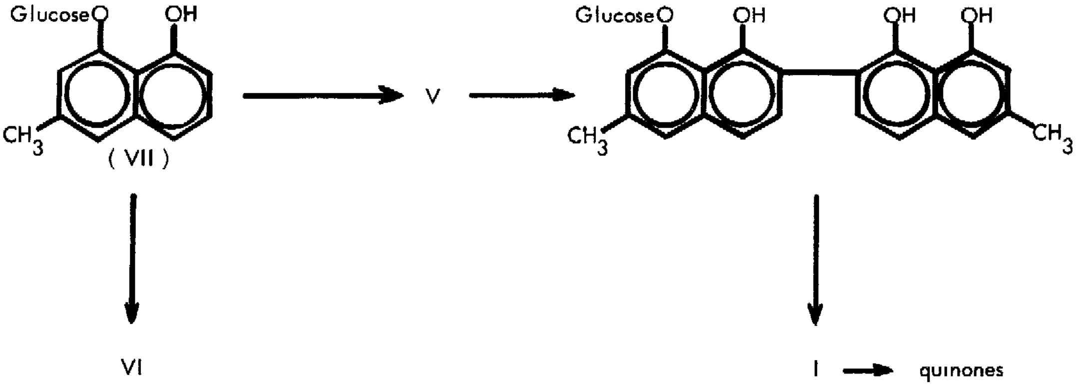
a denotes AB system with J = 9 Hz

b denotes doublet with J = 1.5 Hz

position of proton	II	III	IV
1 and 1' (acetate)	1.93 s	1.93 s	1.92 s
3 and 3'	7.32^{a}	7.33^{a}	7.36^{a}
4 and 4'	7.72	7.65^{a}, 7.74^{a}	7.68^{a}
5 and 5'	7.57^{b} d	7.39^{b}, 7.58^{b} d d	7.41^{b} d
6 and 6' (methyl)	2.46 s	2.47 s	2.47 s
7 and 7'	6.99^{b} d	7.02^{b}, 7.01^{b} d d	6.98^{b} d
8 and 8' (acetate or glucose tetraacetate)	2.31	1.93-2.06 m 3.70-4.33 , 2.30 m s 4.90-5.10 m	1.95-2.07 m 3.70-4.33 m 4.90-5.10 m

One of us had reported previously that the monomer (VI) may be the precursor of diospyrol[5]. There has been considerable controversy concerning the existence of this monomer[3,6]. It can be noted that the monomer was obtained from young berries extracted on the same day of harvest. In our experience, no monomer can be obtained from more mature berries harvested several days before processing as has usually been the case in all chemical studies and therapeutic applications. It is also well known that ripe berries loose anthelmintic efficacy and does not yield diospyrol. Products obtainable by conventional extraction must therefore be considered together with knowledge of spontaneous chemical changes in intact berry as a function of time. Better understanding of the latter has important bearings on both chemical studies and application of the berry in parasite controls.

Results reported here emphasize further the dependence of extractable products on procedure employed. As indicated in the following scheme, we assume that the diglucoside is formed by oxidative coupling of the monomeric glucoside and can,in turn, be converted to diospyrol by successive split of glucose. The major products of any extraction are determined by the age of the berry and conditions employed.



It is not yet known whether the diglucoside has any intrinsic anthelmintic action. Since it can transform into diospyrol, we can conceive that the diglucoside facilitates transport of diospyrol to the parasite by virtue of its greater water solubility. In any case, recognition of the existence of a sequence of reactions extending from intact berry to the intestinal worm should facilitate chemical, pharmaceutical and biological studies of this important berry.

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