Isolation of a New seco-nor-Triterpenol from *Hoya australis* Leaf Wax

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Hoya australis, Asclepiadaceae, Leaf Cuticular Wax, seco-Triterpenoid

From the alcohol fraction of the epicuticular leaf wax of *Hoya australis* R. Br. ex Traill. a new triterpenol was isolated which, according to mass spectral and NMR data, is assumed to be A-seco-A-nor-Δ12-oleanenol.

Introduction

During an investigation of the triterpene composition of cuticular wax on *Hoya australis* leaves in relation to leaf age [1] a striking increase in the concentration of an unknown terpenol was found in relatively old leaves (Compound X-2), but in these the amount of the two triterpenols, lupeol and β-amyrin, which in this order are the main alcohols in young mature leaves, was found to decrease. From experiments with radioactive labelled mevalonic acid it appeared, however, that biosynthesis of lupeol and β-amyrin continues at all age stages. In the unknown terpenol the same precursor is incorporated in relatively old leaves only, although in β-amyrin the amount incorporated remains prominent despite the decrease in its absolute amount (based on leaf surface area) during leaf ageing. This suggested biogenetic relation with β-amyrin together with our interest in the physiological significance of wax triterpenols made identification of the unknown compound necessary.

Material and Methods

^{1}H-FT-NMR spectra were measured in a microcell (5 mm O.D.) in hexadeuterobenzene at ambient temperature with a Varian XL 100-15 spectrometer operating at 100.01 MHz.

GC-MS spectra were recorded both on a JEOL JMS-07 instrument (column glass, 6 ft long, 3 mm I.D. filled with 3% SE-30 on chromosorb WHP 100–120 mesh, column temperature 240 °C, inj. temp. 260 °C) at 70 eV and on a Finnigan 3100 D Gaschromatograph-Mass spectrometer with integrated data system 6100 MS (column 75 cm, glass, 2 mm I.D., 3% OV-1 on chromosorb WHP 100–120 mesh; inj. temp. 250 °C, column temp. 240 °C, pre amp. 10^{-8}, HVS 1.84 kV and beam current 0.10 mA).

High resolution mass measurements were done on a AEI MS 902 mass spectrometer, resolving power 12.000 with an accuracy of 5 ppm.

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Results and Discussion

GC-MS analysis of the terpenol mixture gave the following data for compound X-2: Molecular ion *m/e* 414, and important fragment ions *m/e* 399; 369; 257; 218; 205; 203; 195 and 189 (Fig. 1). For further analysis about 1 mg of X-2 was isolated by GLC. The elemental composition of some of these fragments, determined by high resolution mass measurements, is compared to that of the corresponding fragments of β-amyrin in the following Table:

m/e	X-2	*m/e*	β-amyrin
414	$C_{29}H_{50}O$	426	$C_{30}H_{50}O$
369	$C_{27}H_{45}$	–	–
257	$C_{19}H_{29}$	257	$C_{19}H_{29}$
218	$C_{16}H_{26}$	218	$C_{16}H_{26}$
203	$C_{15}H_{23}$	203	$C_{15}H_{23}$
195	$C_{13}H_{23}O$	207	$C_{14}H_{23}O$

Fragment ions *m/e* 218 and *m/e* 203 of X-2 and β-amyrin are typical for triterpenes with the Δ12-oleanene or ursene skeleton and represent the right hand part of the molecule [2] (Fig. 2). Fragment ion 207 of β-amyrin is characteristic for the left hand part of the molecule. Since the molecular ion of X-2 is one C-atom less than that of β-amyrin, fragment ion 195 must be the one that corresponds to fragment ion 207. As the NMR spectrum of X-2 does not show a signal for vinylic protons, structure analogy with lupeol is not likely (compare below).

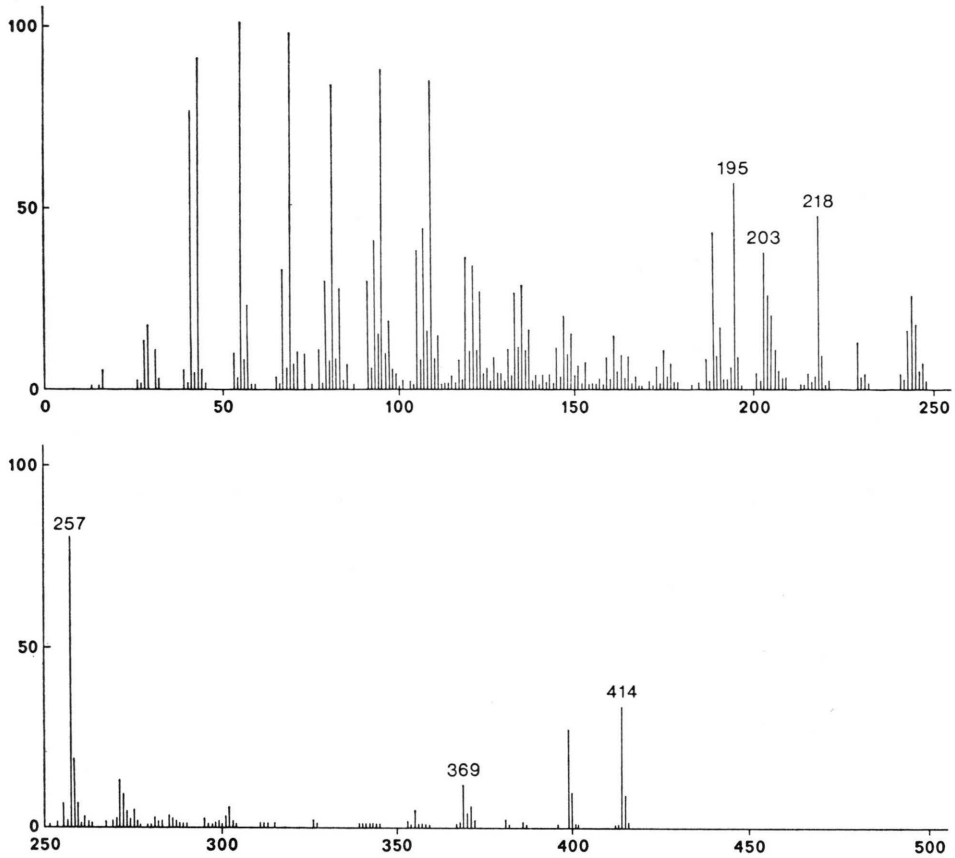

Fig. 1. Mass spectrum of compound X-2.

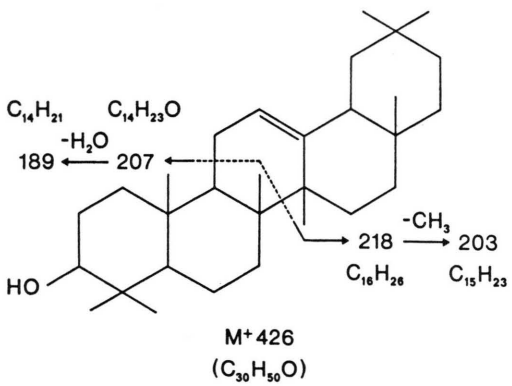

Fig. 2. Fragmentation pattern of Δ12-oleanenes (β-amyrin).

It is evident that the difference must be in the left-hand-side of the molecule which comprises rings A and B. From biogenetic considerations modification of ring A only is probable.

Derived triterpenes can be formed from pentacyclic ring structures by photochemical or oxydative ring cleavage through the loss of one carbon atom, leading to tetracyclic systems with an open A-ring [3 – 6]. Arguments in favour of A-ring cleavage in our case is that, whereas the left hand fragment (*m/e* 207) of β-amyrin is less abundant because of loss of water, the corresponding fragment of X-2 is one of the most abundant fragment ions. This points to a modified structure in the neighbourhood of the hydroxyl group of the molecule, which inhibits the subsequent loss of water.

Acetylation of X-2 gives an acetate with formula $C_{31}H_{52}O_2$, and just as in the case of X-2, the mass spectrum of the acetate showed the fragment ion 369 with the same elemental composition. This last fragment can be formed by cleavage of a $C_4H_7O_2$ fragment of the acetate or a C_2H_5O fragment of the alcohol, and indicates localisation of the hydroxyl group in a short aliphatic side chain.

Nuclear Magnetic Resonance spectra of β-amyrin, lupeol and X-2 are shown in Fig. 3 a – c. A promi-

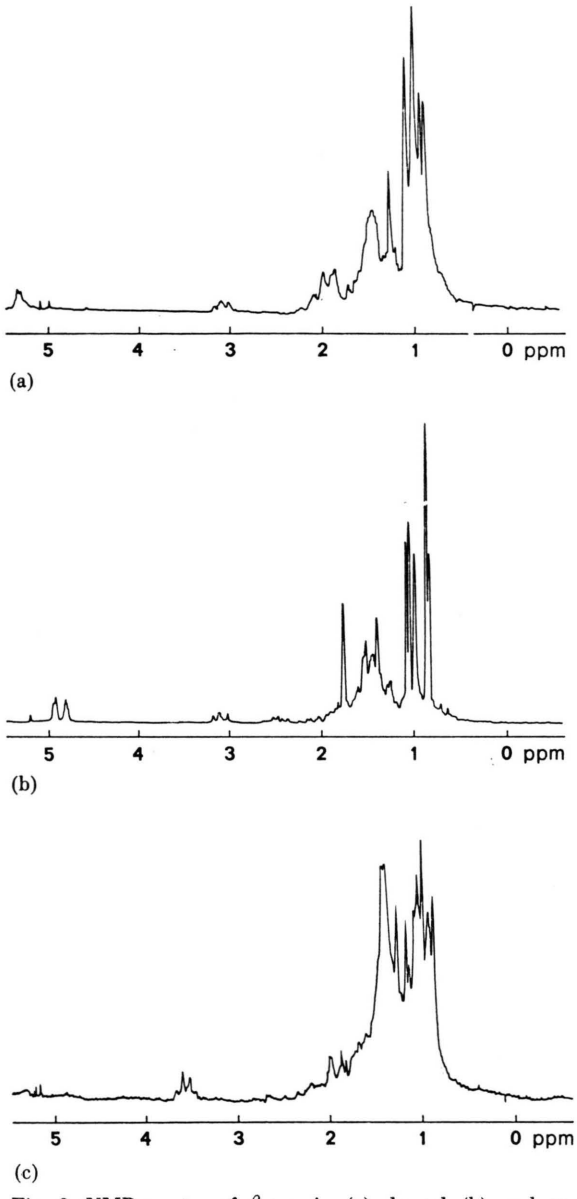

Fig. 3. NMR-spectra of β-amyrin (a), lupeol (b) and compound X-2 (c).

nent feature of the spectrum of X-2, as compared to that of β-amyrin, is the differentiated individual resonances for methyl groups, a considerable integrated area around 1.5 ppm and the presence of a quartet-like multiplet around 3.5 ppm. The latter multiplet could indicate the presence of a methyl group next to the hydroxyl-bearing methylene group. No vinylic proton absorption resembling that of lupeol is found.

All present data strongly suggest that X-2 is a seco-nor-A-triterpenol, for which the partial structure

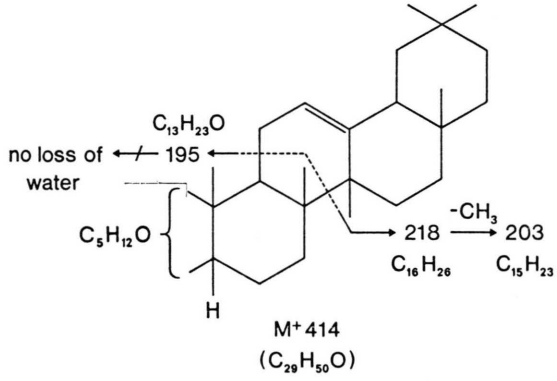

Fig. 4. Fragmentation pattern and a possible structure of compound X-2.

as given in Fig. 4 is postulated. Although seco-A-triterpenols have often been found in nature [7 – 9], this is the first report of a seco-nor-A-triterpenol in living organisms.

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