

Sesquiterpenoid Stress Compounds of *Datura stramonium*: Biosynthesis of the Three Major Metabolites from [1,2-¹³C]Acetate and the X-Ray Structure of 3-Hydroxylubimin

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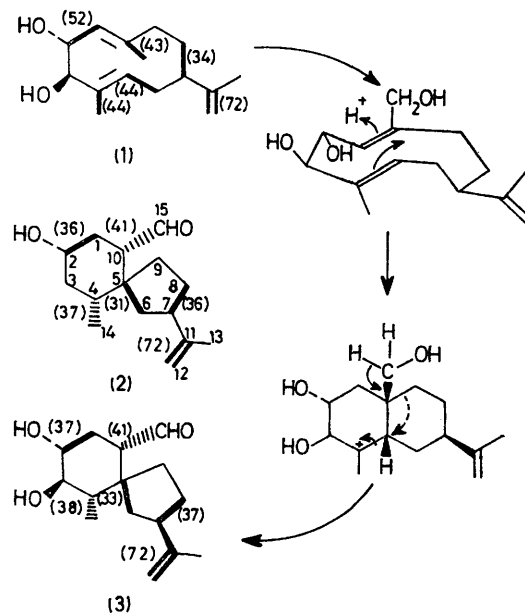
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Summary 2,3-Germacrenediol, lubimin, and hydroxylubimin, the major stress metabolites of *Datura stramonium*, incorporate sodium [1,2-¹³C]acetate in patterns, as shown by ¹³C n.m.r. spectroscopy, which establish their structures and mevalonate origins; the X-ray structure of hydroxylubimin completely defines its stereochemistry.

of silica (t.l.c. type) gave essentially pure germacrenediol (1) (10.9 mg) and two fractions of ca. 10 mg each from which syrupy lubimin (2) (2.4 mg) and crystalline hydroxylubimin

THREE compounds produced by *Datura stramonium* upon infection with fungi¹ were assigned structures (1)—(3) on the basis of their spectra and from biogenetic considerations.^{2,3} These structural assignments have now been confirmed by ¹³C n.m.r. spectroscopic measurements on samples biosynthesized from sodium [1,2-¹³C]acetate. While the spectroscopic data permitted stereochemical assignments^{3,4} for the substituents on the 6-membered ring in lubimin (2) and hydroxylubimin (3), the orientation of the isopropenyl group could not be determined. X-Ray crystallographic results for (3), however, have established this feature and confirmed our earlier stereochemical assignments.

Fruit capsules of the plant were inoculated with spore suspensions of *Monilinia fructicola* as described previously¹ but after 18 h sodium [1,2-¹³C]acetate (90%; 993 mg) and a small amount of sodium [1-¹⁴C]acetate (32 mg) in 200 ml of water were added (0.20 ml per half-capsule); the ¹⁴C-labelled material was included to act as a monitor for the subsequent fractionations. After another 26 h, the liquid in the capsules was collected and extracted with ether. Chromatography of the ether extract (74 mg) on a column



SCHEME

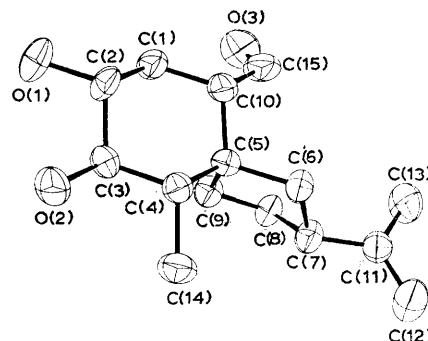
(3) (2.5 mg, m.p. 94–96 °C) were isolated by further chromatography.

The ^{13}C n.m.r. spectra of ^{13}C -labelled compounds (1)–(3) clearly showed that each was formed from three mevalonate units without rearrangement since each spectrum contained twelve signals corresponding to the natural abundance peaks flanked by ^{13}C satellites which were approximately twice as intense as the central signal, and three much more intense signals with relatively small ^{13}C satellites (<6% of the central peak). The latter arose from C-2 of the mevalonate units while the former represent those carbons generated from intact acetate units in mevalonate. The separations of the intense satellites, $J(^{13}\text{C}\text{--}^{13}\text{C})$, in each of the twelve patterns readily allowed pairing of the absorptions for contiguous carbons arising from intact acetate units. These observed coupling constants led to a definite assignment for each signal and ruled out other, possible though less likely, structural formulations for (1)–(3); the $J(^{13}\text{C}\text{--}^{13}\text{C})$ values for the intact acetate units in each skeleton (Scheme) are given in parentheses. In the light of these observations, previous assignments⁴ for C-6 and C-8 and for C-4 and C-7 should be interchanged for (2).† From the intensity ratios (satellites: central peak) for the terminal nuclei (C-12, C-14, and C-15) the incorporation was found⁵ to be 7%. At this relatively high level, the probability of a carbon atom arising from C-2 of mevalonate to be bonded to an enriched carbon atom is significant; thus the ^{13}C satellites for these centres were clearly apparent in the spectra.

3-Hydroxylubimin (3), $\text{C}_{18}\text{H}_{24}\text{O}_3$, m.p. 96.5–99.5 °C, crystallizes in the orthorhombic space group $P2_12_12_1$; $a = 6.190$, $b = 7.210$, $c = 63.082$ Å, and there are two independent molecules in the asymmetric unit ($Z = 8$). Intensity data were collected on a Picker four-circle diffractometer with nickel-filtered $\text{Cu-K}\alpha$ radiation. The structure was solved by direct methods and an E map phased with 381 reflections

with $E \geq 1.4$ revealed all 36 non-hydrogen atoms. At the present stage of the block-diagonal least-squares refinement, $R = 0.094$ for 2301 reflections with $2\theta \leq 130^\circ$.

The structure of (3) is shown in the Figure. The six-membered ring is chair-shaped and all substituents are in an equatorial orientation. The five-membered ring has a half-chair conformation with C-7 and C-8 displaced to the opposite sides of the plane through the other three atoms. The displacements in the two molecules amount to 0.28 and 0.34 Å for C-7 and 0.45 and 0.40 Å for C-8.



FIGURE

The incorporation data for (1)–(3) and the fully established stereochemistry of (3) are consistent with earlier biogenetic proposals^{2,6} as shown for (3) in the Scheme.

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† These atoms correspond to C-1 and C-3, and C-10 and C-2, respectively, in the previous numbering scheme (ref. 4).

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