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

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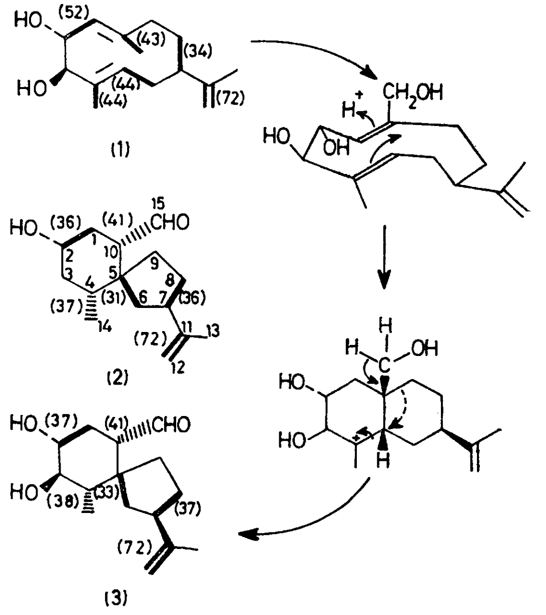
Summary 2,3-Germacrenediol, lubimin, and hydroxylubimin, the major stress metabolites of *Datura stramonium*, incorporate sodium [1,2-^{13}C]acetate in patterns, as shown by ^{13}C n.m.r. spectroscopy, which establish their structures and mevalonate origins; the *X*-ray structure of hydroxylubimin completely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

THREE compounds produced by *Datura stramonium* upon infection with fungi[1] 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 ^{13}C n.m.r. spectroscopic measurements on samples biosynthesized from sodium [1,2-^{13}C]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 previously[1] but after 18 h sodium [1,2-^{13}C]acetate (90%; 993 mg) and a small amount of sodium [1-^{14}C]acetate (32 mg) in 200 ml of water were added (0·20 ml per half-capsule); the ^{14}C-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 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



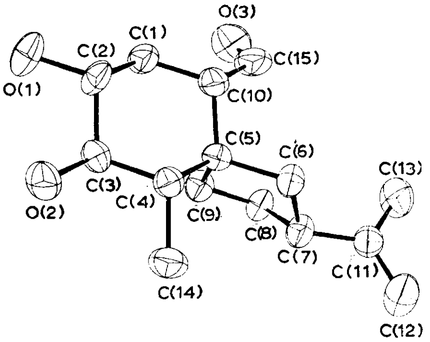
SCHEME

(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, $J(^{13}C-^{13}C)$, 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 $J(^{13}C-^{13}C)$ values for the intact acetate units in each skeleton (Scheme) are given in parentheses. In the light of these observations, previous assignments[4] 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 found[5] 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)**, $C_{15}H_{24}O_3$, m.p. 96·5—99·5 °C, crystallizes in the orthorhombic space group $P2_12_12_1$; $a = 6{\cdot}190$, $b = 7{\cdot}210$, $c = 63{\cdot}082$ 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 Cu-K_α radiation. The structure was solved by direct methods and an E map phased with 381 reflections with $E \geqslant 1{\cdot}4$ revealed all 36 non-hydrogen atoms. At the present stage of the block-diagonal least-squares refinement, $R = 0{\cdot}094$ for 2301 reflections with $2\theta \leqslant 130°$.

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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