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INCORPORATION OF DOUBLY LABELLED SODIUM ACETATE-$^{13}C_2$ INTO PHYTUBERIN AND OTHER SESQUITERPENES IN POTATOES: EXPERIMENTAL CONFIRMATION OF POSTULATED C-C CLEAVAGES

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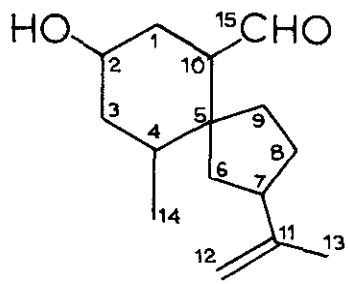
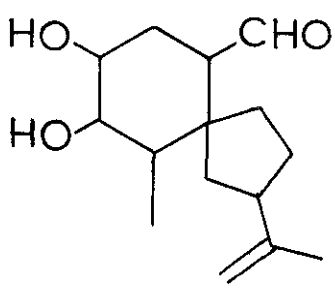
In a continuing study of the sesquiterpenoidal stress metabolites of the Solanaceae, we fed sodium (1,2-^{13}C)-acetate (90% ^{13}C, 1 g) to potatoes (120 lb) which had been inoculated 18 h previously with a spore suspension of Monilinia fructicola. After a further 30 h incubation, chromatography of the ether-soluble material from the diffusates afforded lubimin (1, 16 mg) and hydroxylubimin (2, 7.5 mg); both 1 and 2 showed the same labelling patterns in their ^{13}C n.m.r. spectra as those found for samples previously isolated from the interaction of the fungus with Datura stramonium.[1] Rishitin[2] (3) was obtained as the major metabolite (ca. 70 mg) and its ^{13}C



1



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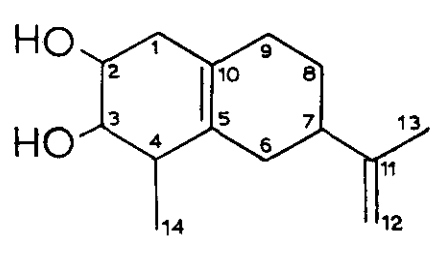

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n.m.r. spectrum (Fig. 1) revealed a labelling pattern consistent with the postulated loss of C-15.[3,4] Disappointingly, the biosynthetically most interesting compound, phytuberin[5,6] (4), was isolated in very small amount (< 1 mg) but some information was obtained from its ^{13}C n.m.r. spectrum in that five signals were clearly discernible, arising from C-1,-2,-3,-9 and -13. This agreed entirely with our expectations since C-3,-9 and -13 arise from C-2 of mevalonate units in an eudesmane precurser while C-1 and -2 arise from an intact acetate fragment, C_4-C_5, of a meva-

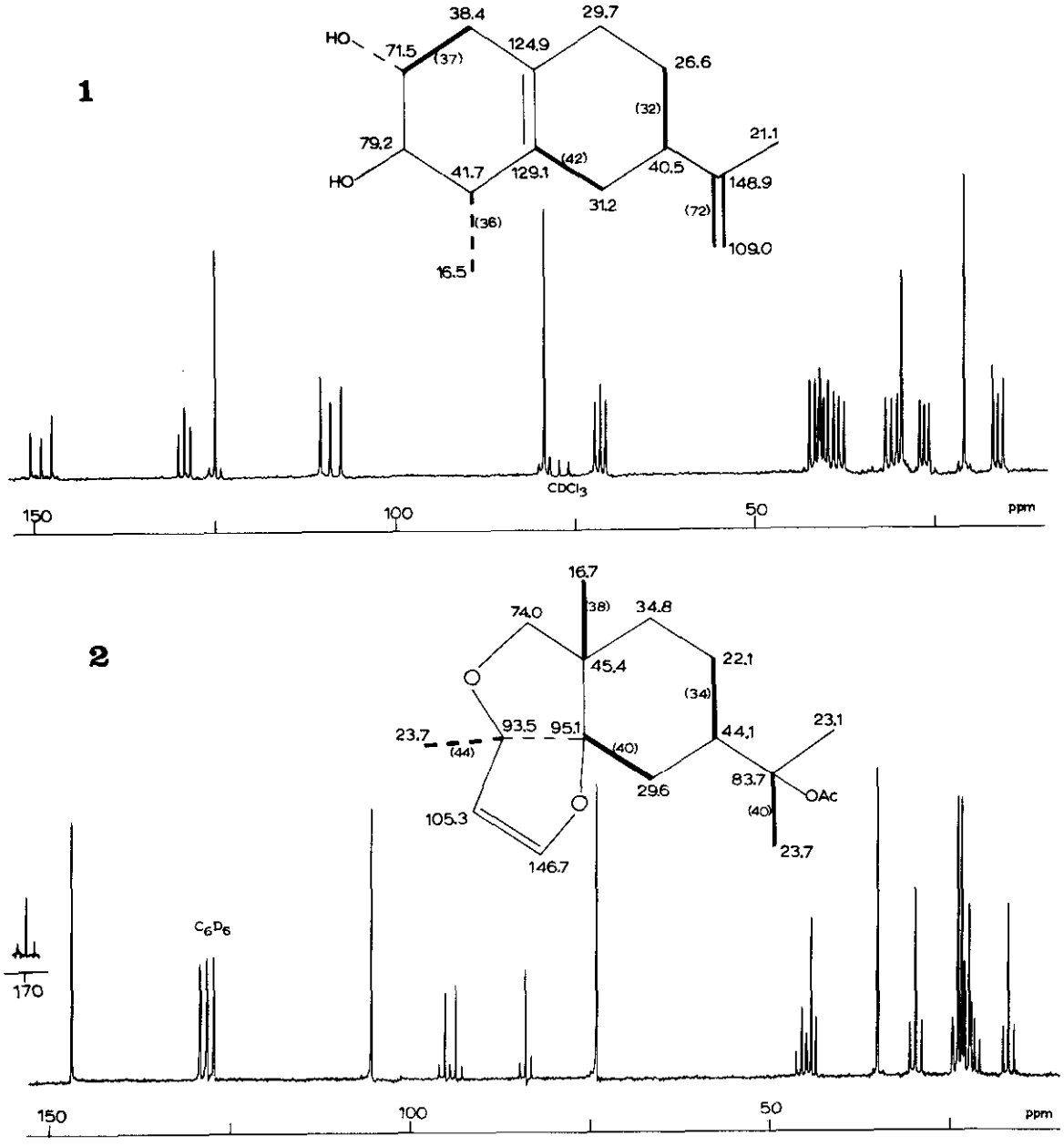
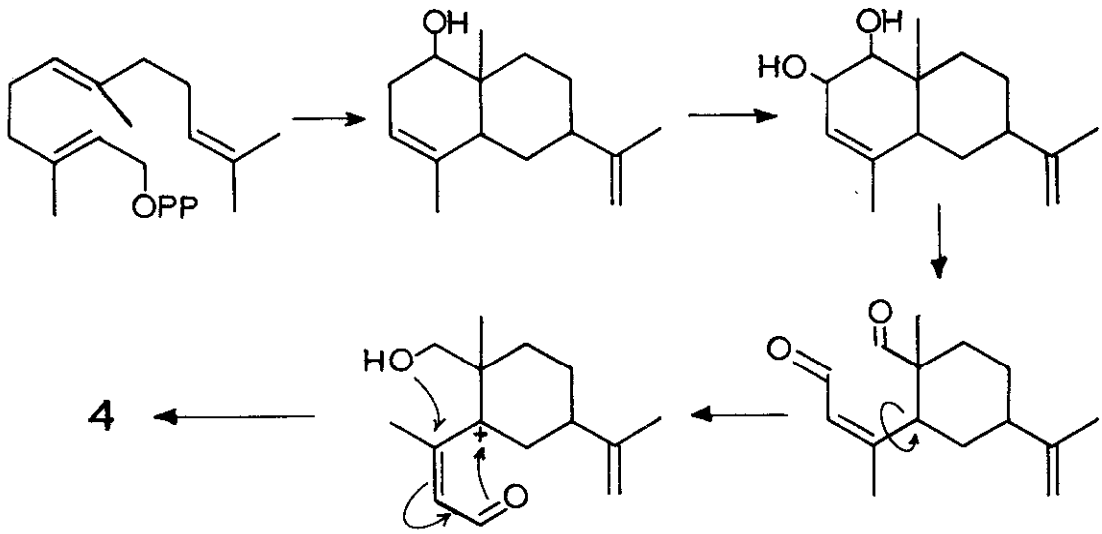


Fig. 1. ^{13}C n.m.r. spectrum of 3-$^{13}C_x$ isolated from the potato - M. fructicola feeding experiment. The individual carbon shieldings are given in ppm from TMS and the J_{C-C} values in Hz (in parentheses) are shown for the pairs of carbons incorporated as intact acetate units.

Fig. 2. ^{13}C n.m.r. spectrum of 4-$^{13}C_x$ isolated from the potato - G. cingulata experiment. The carbon shieldings are given in ppm from TMS and the J_{C-C} values (in parentheses) are those measured for the intact acetate units, indicated by heavy bonds.

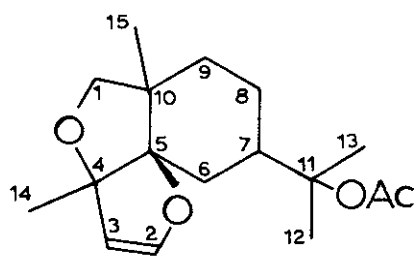
lonate unit which subsequently suffers scission to generate the phytuberin skeleton,[4,6] as illustrated in Scheme 1.

SCHEME 1



A more informative spectrum of 4 was nevertheless desired and for this purpose a similar feeding experiment was performed with Glomerella cingulata as the challenging fungus since this tends to give high yields of phytuberin.[7] In the event, this led to generally lower incorporation levels but the potatoes (64 lb) afforded phytuberin (39 mg) which gave the ^{13}C spectrum shown as Fig. 2. This shows the five intense signals lacking ^{13}C satellites while each of the remaining absorption patterns consists of a central signal flanked by ^{13}C satellites indicating that these arise from acetate units incorporated intact into the skeleton via mevalonate. The separation of each pair of satellite signals is the ^{13}C-^{13}C coupling. With this information one can match the individual signals in pairs and readily complete the signal assignments for each carbon.

The potato - G. cingulata interaction also furnished, in addition to 1, 2 and 3, solavetivone[8] (5) (17 mg) and dihydrolubimin[9,10] (6) (ca. 120 mg), each of which exhibited the ^{13}C



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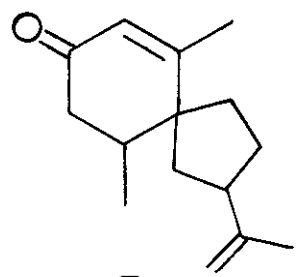

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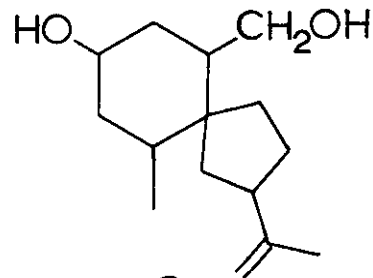

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labelling patterns previously found[1] for 1 and 2, in agreement with expectation. The dihydro derivative 6 probably arose at least in part through the metabolism of 1 by G. cingulata since this process is known to occur in pure culture.[11]

Both feeding experiments afforded additional metabolites on which we shall report in the near future.

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