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BIOSYNTHESIS OF COLCHICINE: INCORPORATION OF A ^{13}C-LABELLED PRECURSOR IN A HIGHER PLANT

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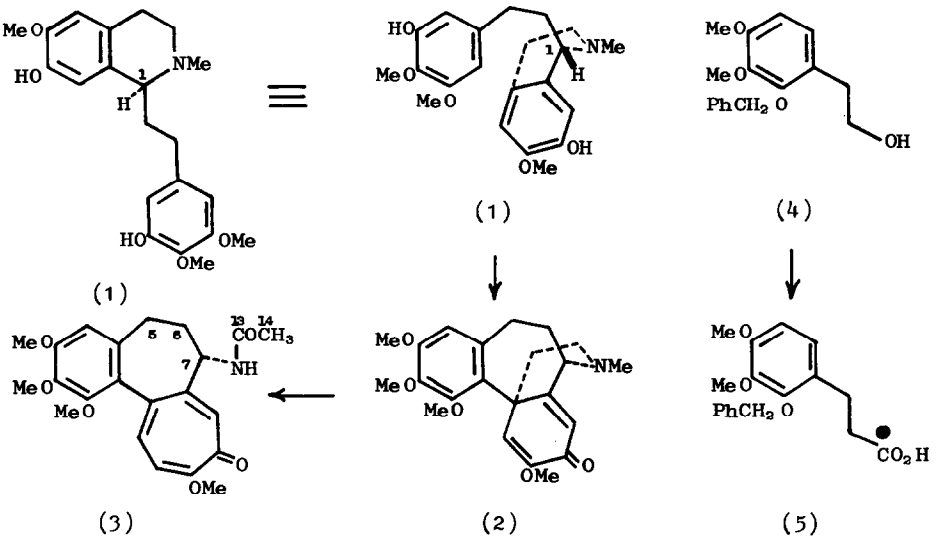
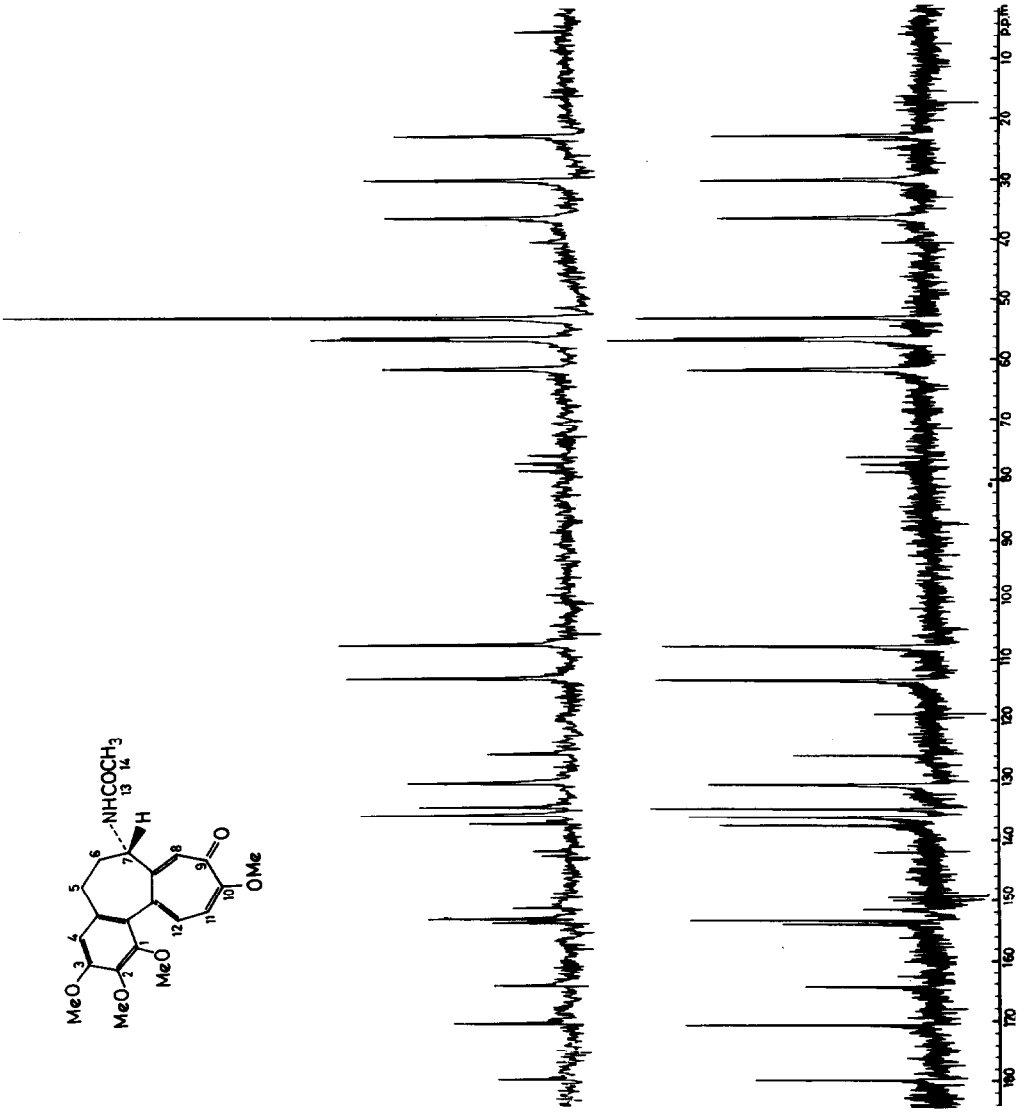
Researches based on carbon-13 labelling combined with ^{13}C-n.m.r. have already proved powerful for the study of biosynthetic pathways.[1] Not only is the labelling pattern directly revealed by the n.m.r. spectrum (provided unambiguous assignments of the signals can be made) but with appropriate design of the experiment, the spectrum can yield additional information of crucial importance.[2] However, the technique has only been used so far with partially purified enzyme systems[2] or with micro-organisms.[3] The difficulty in applying ^{13}C-labelling to studies of higher plants arises from the low




NHCOCH3
13 14
H
MeO
MeO
MeO
OMe
ppm

incorporations that are generally observed and the problem is compounded by dilution of the labelled natural product by unlabelled endogenous material. The dilution can be so large that the ^{13}C-content of the isolated substance does not differ significantly from natural ^{13}C-abundance despite a satisfactory incorporation. However, a dilution of ca. 1:90 or less will give an unequivocal answer provided the precursor is labelled at the level of 90 atom % carbon-13.

TABLE Assignments of ^{13}C-Signals

Chemical shift (ppm)	Assignment	Ratio of integrals, upper spectrum/lower spectrum
22.4	C-6	0.93
29.6	C-5	1.07
36.0	C-14	1.00
52.4	C-7	2.54
55.8	MeO	1.03
56.1	MeO	1.18
61.0	MeO	0.87
61.2	MeO	0.90

A successful experiment is thus only likely in cases where there is (a) a high incorporation or (b) a small pool of the substance of interest. The joint operation of (a) and (b) is clearly ideal and the autumn crocus plant (Colchicum autumnale) seemed to match these requirements. We now report what we believe to be one of the first examples[4] of incorporation of a ^{13}C-labelled precursor in a higher plant.

Colchicine (3) has been proved[5] to be biosynthesised in C. autumnale from the tetrahydroisoquinoline (S)-autumnaline (1) by oxidative coupling followed by ring-expansion of the dienone intermediate (2). Many positions in autumnaline have been labelled with carbon-14 in earlier experiments[6] but not C-1 (corresponding to C-7 of colchicine). (RS)-[1-^{13}C]Autumnaline (1) was prepared by treatment of the O-methanesulphonyl derivative of the alcohol (4) with potassium [^{13}C]cyanide (90 atom % ^{13}C) in dimethylformamide and hydrolysis of the resulting nitrile. The acid (5) was converted as earlier[6]

into (RS)-[1-^{13}C]autumnaline hydrochloride. This (300 mg) was injected as an aqueous solution into the seed capsules of C. autumnale (1 mg per capsule) and after 2 weeks, the capsules were cut off and colchicine (1.24g) was isolated from them.[5]

The lower spectrum in the Fig. is the natural abundance ^{13}C-spectrum of colchicine; the Table gives assignments for the high field signals based on chemical shift and off-resonance decoupling. The upper spectrum is from the enriched colchicine and comparison with the lower one (Table) shows that the signal from C-7 is enhanced ca. 2.5 fold; incorporation (ca. 6%) without randomisation is thus clearly demonstrated. The practicality of studies with multiple labels will be evident.

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