Biosynthesis of Amaryllidaceae Alkaloids. Stereochemistry of Hydrogen Removal α to the Tertiary Nitrogen Atom in the Biological Conversion of Norpluviine into Lycorenine

By **Claudio Fuganti** * and **Mareo Mazza,** Istituto di Chimica del Politecnico Centro del CNR per la Chimica delle Sostanze Organiche Naturali, 20133 Milano, Italy

Feeding experiments with asymmetrically labelled precursors have established that in the conversion of norpluviine into lycorenine the *pro-R* hydrogen atom from C-7 of norpluviine is lost.

THE biological conversion of the Amaryllidaceae alkaloids norpluviine (11) and haemanthamine into lycorenine (13) and haemanthidine, respectively, involves the formal loss of a hydrogen atom from the benzylic position α to the tertiary nitrogen atom. It has been shown [1] recently that the hydrogen which is lost is the one introduced in the protonation taking place during incorporation of 3,4-dihydroxybenzaldehyde (9) into the aromatic C_6–C_1 unit of (11).

We now report the synthesis of and the results of feeding experiments with asymmetrically labelled *O*-methylnorbelladine (10) which establish the absolute stereochemistry of the two related protonation and hydroxylation processes.

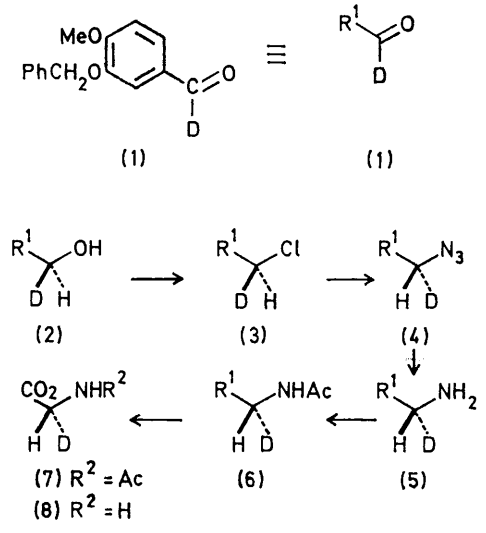
The required stereospecifically labelled precursor (10) was obtained from the optically active alcohol (2) available from the formyl-labelled aldehyde (1) through enzymic reduction (Scheme).[2]

Thus, 3-benzyloxy-4-methoxy[*formyl*-^{2}H]benzaldehyde (1) (89% $^2H_1$ by mass spectrometry) was reduced with liver alcohol dehydrogenase, ethanol, and NADH to the alcohol (2) which was converted by ether–$SOCl_2$ into the chloride (3). This upon treatment with NaN_3 in hexamethylphosphoramide (HMPA) gave the azide (4). Reduction with $LiAlH_4$ of (4) gave rise to the amine (5). Since conversion of (5) into (10) is achieved through a procedure not involving reactions at the chiral centre, the optical purity and the absolute configuration of the

[1] C. Fuganti and M. Mazza, *Chem. Comm.*, 1971, 1196.

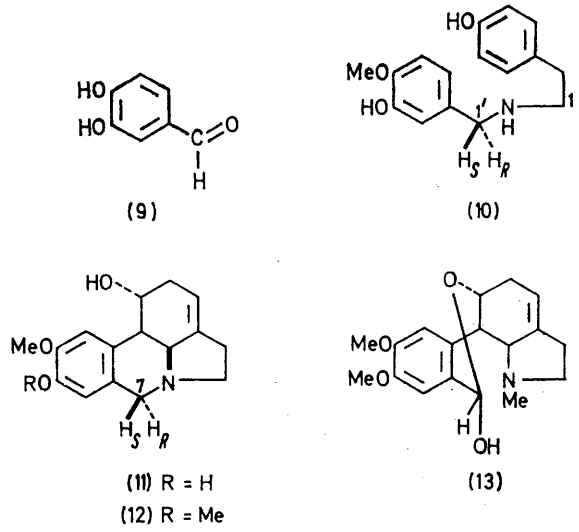
[2] A. R. Battersby, J. E. Kelsey, and J. Staunton, *Chem. Comm.*, 1971, 183 and ref. 6 therein; C. K. Johnson, E. J. Gabe, M. R. Taylor, and I. A. Rose, *J. Amer. Chem. Soc.*, 1965, **87**. 1802.

precursor were determined by correlating (5) with (2*R*)-[2-^{2}H]glycine.[3] This was achieved as follows. Acetylation of (5) with acetic anhydride and pyridine in the cold gave the amide (6) (87% $^2H_1$), from which *N*-acetylglycine (7) was obtained upon ozonolysis and



SCHEME

oxidative work-up. Enzymic hydrolysis of the protecting acetyl group with acylase I afforded [2-^{2}H]glycine. The deuterium content (measured by mass spectrometry on the *N*-benzoylmethyl ester) and o.r.d. measurements indicated that it contained 75 ± 10% of the (2*R*)-isomer (8). The absolute configuration of the major enantiomer of the amine (5) is thus established.



Repetition of the aforementioned sequence starting from the [*formyl*-^{3}H]aldehyde (1) yielded the tritiated amine (5) which was converted into (1′*R*)-[1′-^{3}H,1-^{14}C]-*O*-methylnorbelladine (10).

Texas daffodil incorporated doubly labelled (10) into norpluviine (11) (1·2% incorporation) without loss of ^{3}H. The latter radioactive alkaloid (11) was converted in a later feeding of Inglescombe and Tresamble daffodil into pluviine (12) (11% incorporation) (no loss of ^{3}H), and into lycorenine (13) with 82 ± 5% loss of ^{3}H (4·8% incorporation) thus indicating that in the oxidation of (11) to (13) a *pro-R* hydrogen atom from C-7 is removed.

The present ^{3}H value for lycorenine (13), in the light of the previous results for feeding experiments with (*a*) *O*-methylnorbelladine (10) randomly labelled at C-1′ (*ca.* 50% loss of ^{3}H) and (*b*) [7-^{3}H,5-^{14}C]norpluviine (11) obtained from separate feeding[1] with [*formyl*-^{3}H]-3,4-dihydroxybenzaldehyde (9) and [1-^{14}C]-*O*-methylnorbelladine (10) (no loss of ^{3}H), confirms the stereospecificity of both protonation and hydroxylation processes and, further, establishes that in the incorporation of 3,4-dihydroxybenzaldehyde (9) into the aromatic C_6–C_1 unit of the Amaryllidaceae alkaloids, protonation takes place from the *re*-face[4] of the molecule.

EXPERIMENTAL

Spectroscopic Measurements.—Mass spectra were measured with an Hitachi-Perkin-Elmer RMU-6D double-focusing spectrometer. The o.r.d. measurements of [2-^{2}H]glycine were taken for aqueous solutions on a Cary 60 apparatus at the University of Pisa.

Radioactive Assay.—Activities were measured by liquid scintillation counting with a Packard 3320 instrument, using ^{3}H- and ^{14}C-hexadecane as internal standards. Determinations of activities were made in duplicate.

Synthesis of the Deuteriated Amine (5).—The deuteriated alcohol (2) (890 mg) obtained from 3-benzyloxy-4-methoxy-[*formyl*-^{2}H]benzaldehyde (1) by NADH reduction, was treated in anhydrous ether at room temperature with distilled $SOCl_2$ (4 ml). After 5 h the reaction mixture was evaporated to dryness and the chloride (3) (820 mg) was crystallised from light petroleum. The chloride (3) (820 mg) in HMPA (10 ml) was added dropwise to NaN_3 (1 g) in HMPA (50 ml) with stirring at room temperature. After 24 h 5% aqueous $NaHCO_3$ (100 ml) was added and the cloudy mixture was extracted with benzene–hexane. The organic solvent was evaporated off and the oily residue was purified by chromatography on silica gel with 20% ethyl acetate in benzene. The azide (4) (750 mg) was reduced in boiling ether (100 ml) with $LiAlH_4$ (1 g) to the amine (5) which was isolated by the usual work-up [580 mg, 65% overall yield from (2)].

Conversion of the Amine (5) *into* [2-^{2}H]*Glycine.*—The amine (5) (580 mg) was kept overnight at 5° with acetic anhydride (5 ml) and pyridine (10 ml). The mixture was evaporated under vacuum below 30° and the residue was chromatographed on silica gel with benzene–ethyl acetate (1 : 1) to give the amide (6) (620 mg) which solidified on standing. The amide (6) (620 mg) was dissolved in MeOH–$CHCl_3$ (1 : 1) (30 ml) and the solution was ozonised at room temperature for 8 h; during this time the volume was kept constant by addition of MeOH. The solvent was evaporated off in the cold and after addition of water (4 ml) and then 30% H_2O_2 (1 ml) in 85% formic acid (1 ml), the solution was heated for 1 h on a water-bath. A trace of 10%

[3] P. Besmer and D. Arigoni, *Chimia* (*Switz.*), 1968, **22**, 494.
[4] K. R. Hanson, *J. Amer. Chem. Soc.*, 1966, **88**, 2731.

Pd–C was added and the filtered solution was evaporated to dryness. The residue was taken up in hot MeOH–ethyl acetate and after concentration *N*-acetylglycine (7) crystallised from ethyl acetate as prisms (160 mg, 63%). *N*-Acetylglycine (160 mg) in phosphate buffer (pH 7·4) (20 ml) was kept at 25° for 24 h with acylase I (40 mg). The mixture was then heated on a water-bath for 20 min and filtered (charcoal). [2-^{2}H]Glycine was isolated by chromatography (Dowex 50; 2·5 × 20; H^+) by elution with 2N-NH_4OH and was purified by sublimation and crystallisation from MeOH–H_2O.

Feeding Experiments and Isolation of the Alkaloids.—The precursors were fed by injection of aqueous solutions of the hydrochlorides into the flower stems. The plants were harvested after 10—15 days. Extraction was performed as reported [5] and the alkaloids were separated by alumina chromatography. Several derivatives (picrates, picrolonates, hydrochlorides, and *O*-acetates) were used in the purification to constant activity.

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[5] A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J. Chem. Soc.*, 1964, 1595.