The Biosynthesis of Phenanthrenoid Phytoalexins. Incorporation of Hexadeuteriophenylalanine into Orchinol established by ^{2}H N.M.R. Spectroscopy

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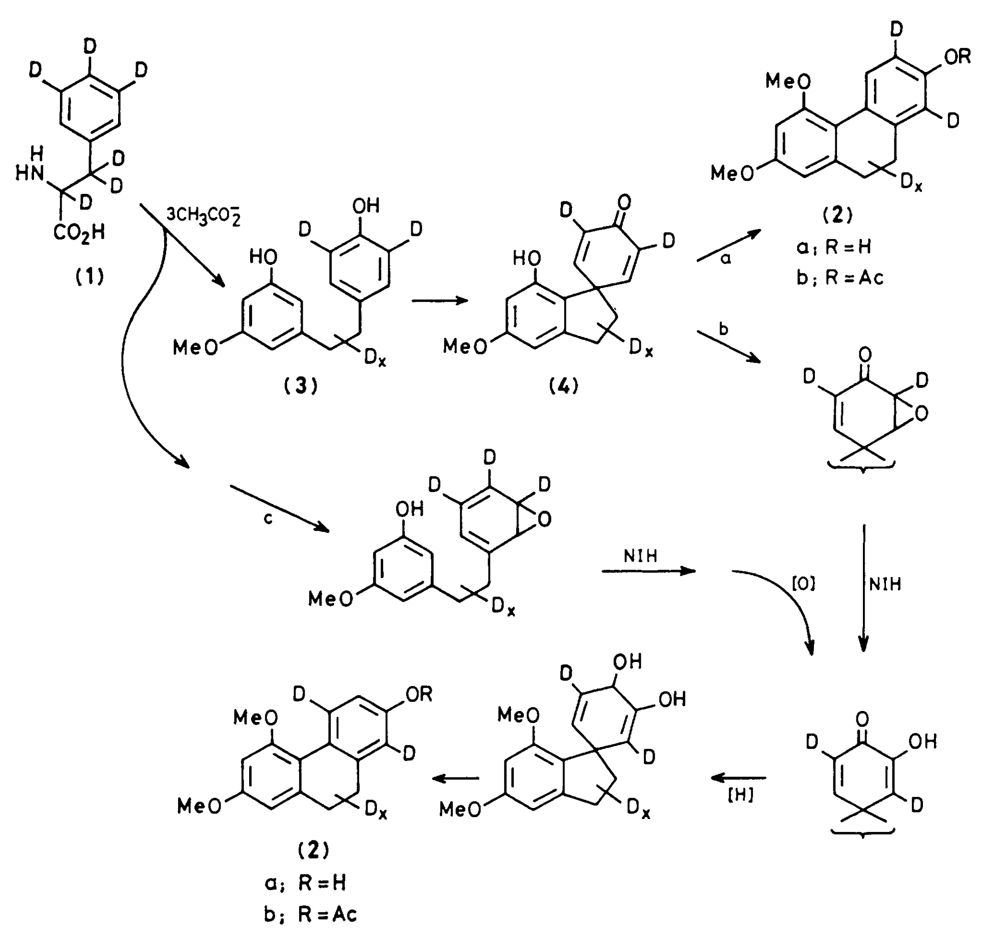
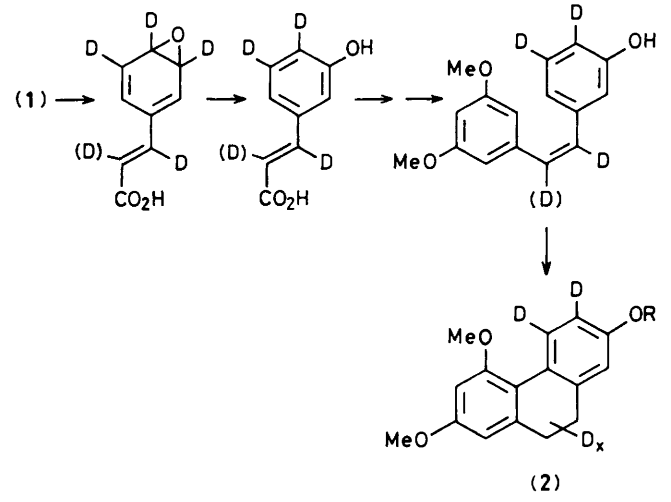
Deuterium n.m.r. studies have shown that α,β,β,3,4,5-hexadeuteriophenylalanine is incorporated into the dihydrophenanthrenoid phytoalexin orchinol with loss of aliphatic deuterium and an unexpected, and as yet unclarified, migration of aromatic deuterium.

The absence of experimental studies on the biosynthesis of naturally occurring phenanthrenes and dihydrophenanthrenes has been noted recently.[1] For this reason it seems opportune to report results obtained for the incorporation of [α,β,β,3,4,5-$^2H_6$]phenylalanine (**1**)[2] into orchinol (**2a**)[3] produced as a phytoalexin[4-6] by *Orchis militaris*.

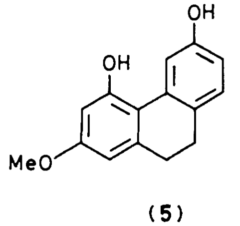
The precursor (**1**) was prepared from L-phenylalanine according to the literature method.[2] Examination by n.m.r. spectroscopy confirmed that this material was deuteriated to $>95\%$ (^{1}H n.m.r.) in the positions specified (^{13}C n.m.r.). Satisfactory feeding conditions were established in preliminary experiments with [U-^{14}C]phenylalanine; these also showed that phenylalanine was a better precursor than tyrosine, cinnamic acid, or *p*-coumaric acid. In the critical experiment, (**1**) (17.3 mg) was administered to sliced tubers (108 g) of the orchid, in which production of (**2a**) had been stimulated by contact, over several hours, with agar plates [1.7% 'Bacto-Agar' (Difco) in H_2O], *i.e.*, with material derived from algal cell walls. After six days in the dark on the agar at 24 °C, the tuber slices and agar were extracted with EtOH, the extracts were concentrated, and the EtOH-soluble portion of the product was chromatographed (Kieselgel 60; MeOH–$CHCl_3$, 2:98). The orchinol-enriched fraction so obtained was acetylated (Ac_2O–pyridine) and rechromatographed over silica gel (Camag DF5) in Et_2O and again in CH_2Cl_2–$CHCl_3$ (1:1) to give orchinol acetate (**2b**) (14.0 mg). The ^{2}H n.m.r. spectrum of (**2b**) contained three signals at δ 8.25 (^{2}H-5), 6.94 (^{2}H-6 and/or -8), and 2.72 p.p.m. (^{2}H-9 and/or -10) with relative areas in



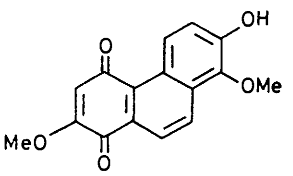
Scheme 1



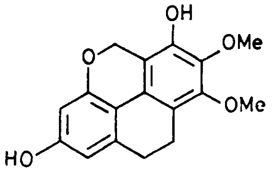
Scheme 2



(5)



(6)



(7)

the ratio 0.5 : 0.75 : 1.0. This pattern is incompatible with the sequence shown in Scheme 1, path a, with direct conversion of (**4**) into (**2**), which was originally favoured[6] on mechanistic grounds and because it is in excellent accord with the co-occurrence of (**3**) (D = H), (**4**) (D = H), and (**5**) in *Cannabis sativa*.[7] The observed spectrum, however, is consistent with the formation of (**2a**) through Schemes 1 paths b and c or 2. Scheme 1, path b differs only in oxidation sequence from that originally proposed by Birch;[8] Scheme 1, path c is equivalent but, perhaps, mechanistically more attractive.

We must point out, however, that none of these Schemes is fully satisfactory when considered in the light of the co-occurrence of the *Cannabis* metabolites noted above or the oxygenation pattern of cypripedin (**6**) from the orchid *Cypripedium calceolus*[10] and 6,7,8-trioxydihydrophenanthrenes such as (**7**), recently isolated from the orchid *Coelogyne cristata*.[11] In addition we know of no precedent for the type of NIH shift depicted in Scheme 1b. Also, it is known that the corresponding shift does not occur during the enzymic hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine.[9a] However, an epoxide analogous to the 2,3-epoxide depicted in Scheme 1c has been postulated as an intermediate in the biosynthesis of gliotoxin.[9b] Thus, while our results provide conclusive evidence that rings B and C of orchinol (and hence, presumably also those of certain other naturally occurring phenanthrenes[12]) are derived from phenylalanine as previously predicted, they also suggest that the precise pathway is more complex than hitherto envisaged.[6-8]

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