

INCORPORATION OF L- [U-¹⁴C]-β-PHENYLALANINE
INTO THE LIGNAN PODOPHYLLOTOXIN

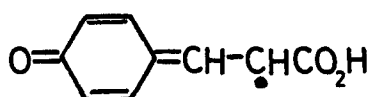
D.C. Ayres

Westfield College, University of London,
Hampstead, London, N.W.3.

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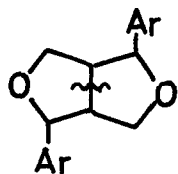
The lignans (1) form an extensive group of naturally-occurring phenols and Erdtman (2) first suggested that they were formed in vivo by the oxidative coupling of two cinnamate residues: a view which is now widely held. The first experimental evidence in accord with this postulate is reported here.

The analogous in vitro coupling of mesomeric cinnamate radicals (I) is well established (3) and Freudenberg and his colleagues (4) have isolated racemic lignans (e.g. II) during demonstrations of oxidase activity in lignin synthesis. In two instances (5) racemic lignans have

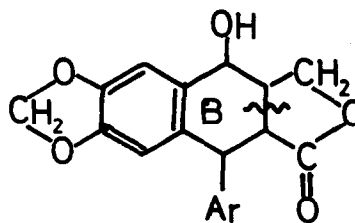


I

↓ cinnamate



II



III Ar = 3,4,5-trimethoxyphenyl

been isolated together with lignin, nevertheless as they occur typically lignans are optically active and a direct link with lignin biogenesis cannot therefore be assumed (6).

Only one example is known (7) of the co-occurrence of enantiomeric aryl naphthalene lignans. Of this class podophyllotoxin (III) was selected for study because of its pharmacological importance (8) and the ease of cultivation in England of the source plant, Podophyllum emodi WALL. Plants grown in pots for four years were not satisfactory since the root and rhizome (ca 2.5 g) afford only limited amounts (ca 7 mg) of the lignan; two five-year plants grown in the open were therefore used for the work. It was known (9) from autoradiography experiments that 4 days elapsed before label from wick-fed [$U-^{14}C$]-phenylalanine (15 mc/mM) could be detected in untreated petioles and metabolism of the nutrient (5 μ c/plant) was therefore extended to 8 days. After harvesting, the podophyllin resin (295 mg) was isolated, reprecipitated (269 mg) and worked up by chromatography on alumina (8); the podophyllotoxin isolated (96 mg) was characterised by m.p., t.l.c., and its infrared spectrum (KBr disc) (8).

Radiochemical Counting

A Panax Ltd. coincidence control unit 2032 with liquid measuring head 2022, automatic timer T300, and counter D 657 were used. The samples (ca 3 mg) were dissolved in ethanol (1 ml) and scintillations counted in toluene [10 ml, containing p-terphenyl (30 mg) and 1,4-bis-2(5-phenyl-oxazolyl) benzene (1 mg)]; quenching was evaluated as in a preliminary study (10) -

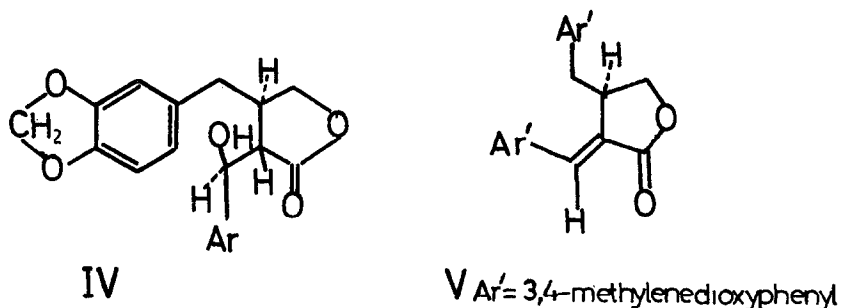
Podophyllin resin had 23.0 d.p.s./mg

Podophyllotoxin had 54.2 d.p.s./mg,

The level of incorporation (1.4%) in the lignan is high enough to permit further study of its seasonal turnover in the plant (11). Experiments

with specifically labelled precursors should determine whether, or not, podophyllotoxin biosynthesis follows from the coupling of two C6-C3 residues wholly derived from phenylalanine.

Further investigation (12) of *P. emodi* lead to the isolation of podorhizol (IV), which suggests that the B-ring of its congener, podophyllotoxin (III), is closed by a Friedel-Crafts reaction. A biogenetic relationship between these compounds is also likely in view of the fact that savinin (V), which co-occurs with podophyllotoxin in *Juniperus spp.* (13), has the same absolute configuration as anhydropodorhizol.



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