Biosynthesis of the Rotenoid Amorphigenin in Germinating *Amorpha fruticosa* seeds: the Post-isoflavone Stages

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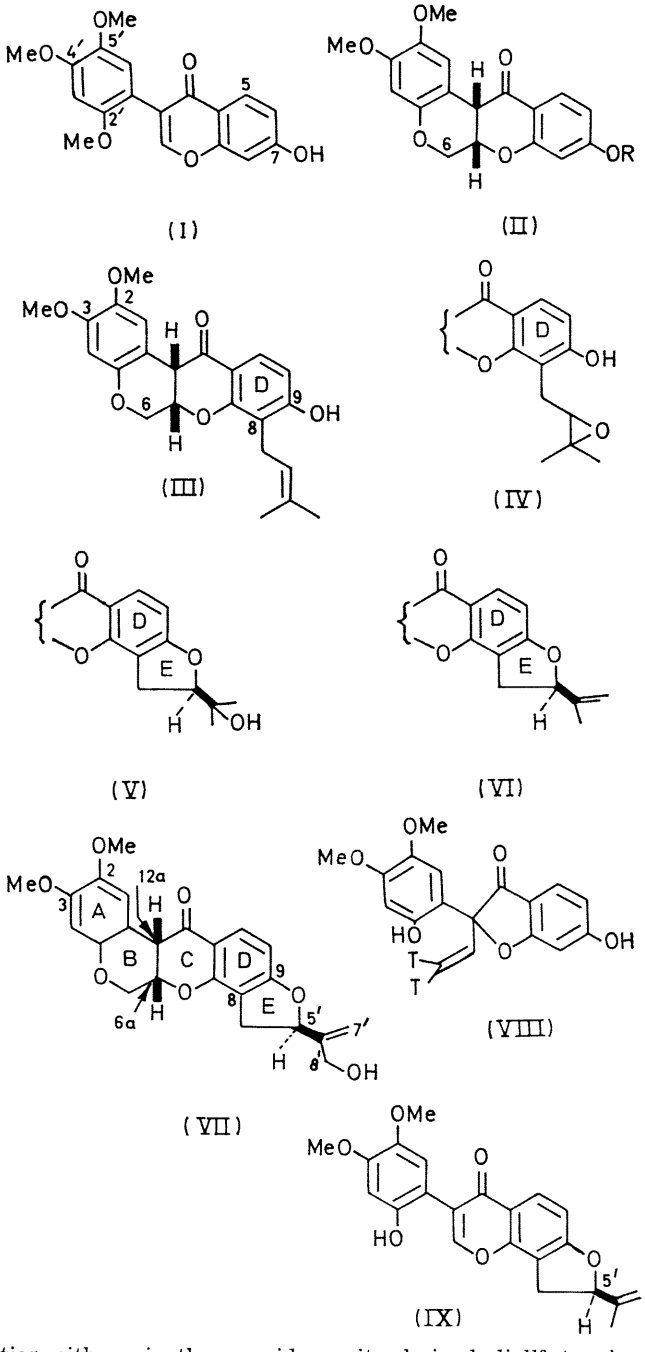
Summary (±)-[6-^{3}H]-9-Demethylmunduserone (II; R=H) is a good precursor for amorphigenin in *A. fruticosa* and holds a key biosynthetic position; incorporation of (±)-[6-^{3}H]rotenonic acid (III), and [6-^{3}H]mutarotenone into amorphigenin lead to a scheme for the ring-E prenyl elaboration.

A DIRECT experimental link between 2′-methoxyisoflavonoids and rotenoids has recently been demonstrated by the biosynthetic conversion of (I) into amorphigenin (VII) by sterile germinating seeds of *Amorpha fruticosa*.[1] The isoflavone (I), however, when carrying the E-ring attachment of amorphigenin or rotenone, is not an acceptable precursor and the conclusion may be drawn that prenylation is a later phase in rotenoid biosynthesis. This places 9-demethylmunduserone (II; R=H) in a key position, for if it is not trapped as the natural rotenoid munduserone (II; R=Me),[2] it becomes the apparent parent of that sub-family of rotenoids having 2,3-dimethoxylation in ring A, resorcinol oxygenation in ring D, and ring E formed from an elaborated or degraded prenyl residue (*e.g.* rotenone, dalpanol, amorphigenin, deguelin, elliptone *etc.*). 9-Demethylsermundone[3] and the 2,3-methylenedioxy-analogue of this, and of 9-demethylmunduserone, may be accorded similar places as heading other rotenoid sub-families.

To confirm the role of 9-demethylmunduserone, the compound has now been synthesised in (±)-form, tritiated at C-6, by using our recent rotenoid synthesis.[4,5] Tritiated dimethylsulphoxonium methylide was used to prepare the intermediate vinylcoumaranone (VIII) which was rearranged[4] to [6-^{3}H]-(II). Administration of the latter to germinating *Amorpha* seeds resulted in a very satisfactory conversion into amorphigenin (1·14%, Table).

Attempts to study the biosynthetic prenylation of (II) were frustrated by unconvincing incorporations of [2-^{14}C]-mevalonic lactone into amorphigenin (*ca.* 0·0003%; cf. [2-^{14}C]acetate 0·22%).[6] Either compartmentation is being encountered or the precursor is unacceptable (*cf.* similar difficulties in other phenolic systems).[7] For the present, the difficulty was circumvented. Rotenone (VI) was prepared, tritiated at C-6, from the corresponding 5′-*R*-isoflavone (IX) using the tritiodimethylsulphoxonium methylide method. This reaction gives a mixture of two diastereoisomers, ("mutarotenone")[8] 6a*S*, 12a*S*, 5′*R* (natural) and 6a*R*, 12a*R*, 5′*R*. 1,4-Hydrogenolysis in ring E then yielded (±)-[6-^{3}H]- rotenonic acid (III),[9] the desired 8-prenylated compound. Administration to the seed system showed good conversion (0·76%) into amorphigenin (VII). [6-^{3}H]Rotenone (VI), administered as 'mutarotenone' was also converted (1·00%) into amorphigenin.

These observations suggest that the post-isoflavonoid stages in amorphigenin biosynthesis are likely to be: formation of 9-demethylmunduserone (II; R=H), its 8-dimethylallylation (III), epoxidation (IV), and cyclisation either *via* the epoxide or its derived diol[10] to give dalpanol (V), recently discovered in Nature.[11] Dehydra-



TABLE

Incorporation of precursors into amorphigenin by germinating Amorpha fruticosa *seeds*[a]

Precursor	Incorporation (%)	Dilution	Uptake (%)
(±) [6—^{3}H] 9 Demethylmunduserone (II R=H)[b]	1 14[d]	244[d]	70
(±) [6—^{3}H]Rotenonic acid (III)[b]	0 76[d]	293[d]	67
[6—^{3}H]Rotenone (VI)[c e]	1 00[e]	320[e]	57
[2′—^{14}C methoxy]Isoflavone (I)[b]	1 81	158	70

[a] Administration period 48 h
[b] Sodium salt in phosphate buffer, pH 7 0
[c] In ethylene glycol monomethyl ether–Tween 20–sterile water
[d] Corrected for utilisation of one enantiomer
[e] Administered as 'mutarotenone' and corrected arbitrarily for utilisation of one diastereoisomer in a 50 50 mixture [8]

tion then gives rotenone (VI) and 8′-hydroxylation of the latter leads to amorphigenin (VII) A hydroxylation of this type is known in the detoxification of rotenone by mammals and insects [12]

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[1] L Crombie P M Dewick, and D A Whiting, *Chem Comm* , 1970, 1469
[2] N Finch and W D Ollis, *Proc Chem Soc* , 1960, 176
[3] For sermundone see W D Ollis in Proceedings of the Symposium on Phytochemistry Sept. 11—16, 1961 ', p 128, ed. H. R. Arthur Hong Kong Univ Press
[4] L Crombie, P W Freeman, and D A Whiting, *Chem Comm* , 1970, 563
[5] We appreciate the advice of Mr P W Freeman on this preparation
[6] Low incorporations of mevalonic lactone into rotenone in *Derris elliptica* plants have been experienced in our earlier work (*e g.* M. B Thomas Ph D Thesis, London, 1965) and in a report by M Hamada and M Chubachi, *Agric and Biol Chem* (*Japan*), 1969, **33**, 793
[7] S A Brown, *Phytochemistry*, 1970, **9**, 2471
[8] L Crombie, P J Godin, D A Whiting, and K S Siddalingaiah, *J Chem Soc* , 1961, 2876
[9] H L Haller and P S Schaffer, *J Amer Chem Soc* , 1933, **55**, 3494
[10] M El Dakhakhny, W Steck, and S A Brown, *Canad J Biochem* , 1970, **48**, 863, W. Steck and S A Brown, *ibid* , 872, R M Bowman, J F Collins, and M F Grundon *Chem Comm* , 1967, 1131
[11] D Adinarayana, M Radhakrishniah, J Rajasekhara Rao, R Campbell, and L Crombie, *J Chem Soc* (*C*), 1971, 29
[12] J. Fukami, I Yamamoto and J E Casida, *Science*, 1967, **155**, 713