Identification of "Metabolite C" from Abscisic Acid and a New Structure for Phaseic Acid

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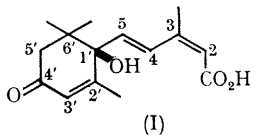
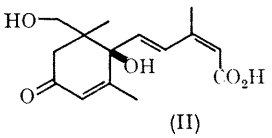
Summary (+)-Abscisic acid is metabolized to the (+)-hydroxymethyl compound (II), which rearranges to the oxabicyclo[3,2,1]octane derivative (III) on methylation.

WHEN (±)-[2-^{14}C]abscisic acid[1] was metabolized by tomato (*Lycopersicon exculentum*) shoots[2] it was rapidly converted into [^{14}C]abscisyl-β-D-glucopyranoside.[2] The remaining abscisic acid (ABA) contained an excess of the (−)-enantiomer, as did the ABA released by hydrolysis of the glucose ester.[3] The balance of the radioactive (+)-ABA (I) was converted into an acidic compound, previously designated "Metabolite C",[4] which was the only other radioactive material found in significant amounts.

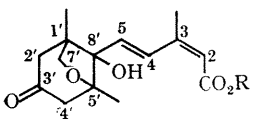
"Metabolite C" has now been identified as (II). Methylation gave an ester which rearranged rapidly to (III). Direct comparisons of this material with phaseic acid methyl ester showed that the rearranged "Metabolite C" is identical with phaseic acid, for which the tentative structure (IV) was suggested by MacMillan and Pryce.[5]



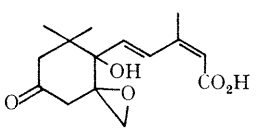
(I)



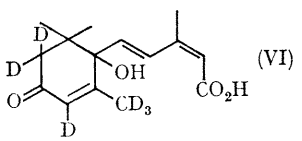
(II)



(III) R=Me
(V) R=H



(IV)



(VI)

The u.v. and o.r.d. spectra of abscisic acid and "Metabolite C" were almost identical, while the molecular ion in the mass spectrum of "Metabolite C" contained one oxygen atom more than that of ABA (this mass spectrum was later attributed to a rearrangement product). The presence of absorption bands at 1585, 1615, and 1666 cm.$^{-1}$ in the i.r. spectrum of (II) and the similarity of the strong positive Cotton effect centred at 274 nm. to that of ABA indicated that the double bonds in the ring and the side-chain of ABA were still present. The hydroxy-group absorption at 3400 cm.$^{-1}$ was more intense than that of ABA. "Metabolite C" reacted with acetic anhydride in pyridine to give a less polar product; the tertiary hydroxy-group of ABA cannot be acylated in this way. "Metabolite C", therefore, is a hydroxylated derivative of ABA. The metabolite failed to react with ethereal periodic acid, as shown by recovery of unchanged material. Disregarding skeletal rearrangement, these properties leave only the four methyl groups as possible sites for a new hydroxy-group.

The crystalline rearrangement product has a plain, negative o.r.d. curve between 250 and 350 nm. and the hydroxy-group absorption is less than that of "Metabolite C"; an absorption band at 1715 cm.$^{-1}$ had appeared while the one at 1666 cm.$^{-1}$ disappeared on rearrangement. These changes were attributed to the saturation of the double bond $\alpha\beta$ to the keto-group. The n.m.r. spectra of the methyl ester of the rearrangement product in deuteriochloroform and hexadeuterioacetone showed that the 3-methylpentadienoic acid side-chain of ABA[6] was unchanged, so the hydroxylation must have occurred on one of the methyl groups on the cyclohexene ring of ABA.

The mass spectrometric fragmentation pattern, i.r., u.v., o.r.d., and n.m.r. properties were identical with those reported for phaseic acid methyl ester[6] and the material derived from tomatoes co-chromatographed in four solvents with a sample of methyl phaseate prepared from phaseic acid which had been provided by Dr. MacMillan.

The rearranged "Metabolite C", therefore, is phaseic acid (V).

The structure (III) also provides an explanation for the long range coupling (J 2·0 Hz.) observed[6] between one of the protons responsible for a signal at δ 2·51 (attributed to protons on C-2′) and a proton in a complex signal centred at δ 3·86 (C-7′).

That one of the C-6′ methyl groups of ABA rather than the C-2′ methyl was hydroxylated to form "Metabolite C" was confirmed when hexadeuterio-ABA was supplied to tomato shoots. The deuterio-ABA (V) was prepared by base-catalysed exchange with 1N-NaOD in D_2O, and the positions of the deuterium atoms were determined by comparison of the n.m.r. and mass spectra with those of ABA, isophorone, and octadeuterioisophorone prepared in the same way. N.m.r. and mass spectrometry of phaseic acid derived from this deuteriated ABA showed that some deuterium had been lost from C-2′ and C-4′ (C-5′ and C-3′ of ABA) but the n.m.r. peak at δ 1·23, attributed to the C-5′ methyl of (III), was absent as expected. This correlated the C-5′ methyl of phaseic acid with the δ 1·23 signal which has an integral of 3 protons in undeuteriated material and it could not, therefore, be the site of hydroxylation. The integral of the peak at δ 2·51 of methyl deuteriophaseate was less than that at δ 2·63 as predicted from their assignment to the protons on C-2′ and C-4′, respectively.

The retention of all the deuterium atoms of the C-5′ trideuteriated methyl from (VI), together with the loss of the signal of one of the *gem*-dimethyl groups present in ABA and the occurrence of a new CH_2 signal in the n.m.r. spectrum of methyl phaseate, confirmed that "Metabolite C" was formed by the hydroxylation of one of the C-6′ *gem*-dimethyl groups of ABA.

Whether the hydroxymethyl group is *cis* or *trans* to the tertiary hydroxy-group is at present under investigation.

The mass spectrum of phaseic acid does not differ significantly from that of "Metabolite C". It is concluded,

therefore, that the latter rearranged in the mass spectometer The behaviour of the acicular crystals of "Metabolite C", which melted at 190° and immediately recrystallised at hexagonal plates which melted at 204—205° (Kofler block), also suggests that heating causes rearrangement.

The new structure (V) for phaseic acid accounts for its relative stability to alkali, noted by MacMillan and Pryce[5] as anomalous for structure (IV). The rearrangement of "Metabolite C", a nucleophilic addition to an $\alpha\beta$-unsaturated ketone, may occur *in vivo* or phaseic acid may be an artefact of extraction. Phaseic acid has less than 1/200th of the growth-inhibitory activity of abscisic acid in the wheat-embryo germination bioassay.

I acknowledge the help of Professor J. W. Cornforth, who suggested the new structure for phaseic acid.

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[5] J. MacMillan and R. J. Pryce, *Chem. Comm.*, 1968, 124.
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