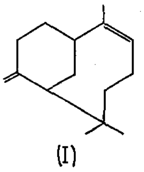
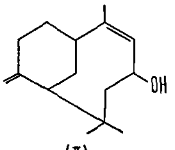
This alcohol, $[\alpha]_D + 19.1°$, was isolated in a homogeneous state with respect to gas chromatography by means of the combination of fractional distillation and elution chromatography (silica gel and n-hexane involving 15% ethyl acetate). It has a molecular formula of $C_{15}H_{24}O$ upon the determination of the molecular ion (m/e calcd. 220.183, obsd. 220.184), and exhibited IR-absorption bands (liquid film) and NMR-signals (in $CDCl_3$) attributable to a hydroxyl group and a proton on the carbon atom bearing a hydroxyl group respectively at 3300 and 1019 cm^{-1}, 1.56 ppm (1H, exchangeable with D_2O) and 4.15 ppm (1H, m). When the compound was treated overnight with acetic anhydride in pyridine at room temperature, it easily produced an acetylated product which showed characteristic signals at 2.08 ppm (3H, s, $-OCOCH_3$) and 5.40 ppm (1H, m, $H-\overset{|}{\underset{|}{C}}-OAc$). On the basis of these evidences the compound was characterized as a secondary alcohol.

NMR- and IR-spectra of this alcohol also indicate the presence of an exomethylene group (1645 and 888 cm^{-1}, 4.87 and 5.06 ppm, each 1H and s), a partial structure of $CH_3-\overset{|}{C}=\overset{|}{C}-H$ (823 cm^{-1}, 1.80 ppm, 3H, d, J = 1.5 Hz, 5.49 ppm, 1H, broad d, J = 5.0 Hz) and one geminal dimethyl group (1382 and 1377 cm^{-1}, 0.90 and 1.06 ppm, each 3H and s). All these groups were recognized in the previously reported hydrocarbon, bazzanene, and the whole pattern of the NMR-spectrum, excepting signals related to the hydroxyl group, resembles closely the spectrum of bazzanene, although 2 signals of the vinyl proton and of the methyl group attached to the double bond are both split into doublets in this alcohol. Such spectrometric resemblance between bazzanene and bazzanenol, and the above fact that the vinyl proton signal is split into doublet, make us assume bazzanenol to be a secondary alcohol which has a hydroxyl group at C_4-position of bazzanene.

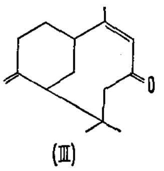
In order to give the positive proof for this assumption, the alcohol was oxidized with Jones reagent, and an α,β-unsaturated ketone, $C_{15}H_{22}O$ (m/e calcd. 218.167, obsd. 218.166, ν_{max}^{liq} 1672 cm^{-1}, $\delta_{ppm}^{CDCl_3}$ 6.50, 1H, m) was obtained. These IR-, NMR- and mass-spectra of this ketone coincided respectively with those of a ketone (III) derived from bazzanene by sodium dichromate oxidation[2].



(I)



(II)



(III)

Zusammenfassung. Isolierung und Strukturaufklärung eines neuen Sesquiterpenalkohols aus *Bazzania pompeana* (Lac.) Mitt.

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Hiroshima (Japan), 28 October 1969.

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[1] Studies on chemical constituents from Hepaticae, Part III: S. HAYASHI, A. MATSUO and T. MATSUURA, Tetrahedron Letters, *1969*, 1599 and ref. [2] are regarded as Part I and II.

[2] S. HAYASHI, A. MATSUO and T. MATSUURA, Experientia *25*, 1139 (1969).

The Biosynthesis of Trisporic Acids from β-Carotene via Retinal and Trisporol

The trisporic acids, obtained from 'mated' cultures of *Blakeslea trispora*, include the 9-*cis* and -*trans* isomers[1] of trisporic acid C (Ia)[2], the main component, and of the corresponding 13-keto-compound trisporic acid B (Ib). The acids promote carotene and steroid synthesis in (–)-*B. trispora*[3], apparently by an enzyme-induction mechanism[4]. Moreover the 'gamones' which, in heterothallic Mucorales generally, diffuse between opposite mating types and elicit sexual differentiation, have been identified with trisporic acids[5]. In particular, the active extract from mated *Mucor mucedo* has afforded identifiable *cis*- and *trans*-trisporic acids A, B, and (principally) C, and conversely pure methyl trisporate-B and trisporate-C have activity towards (–)-*M. mucedo* which is quantitatively comparable to that of the total extract from mated strains of the same fungus[6].

Amongst the many co-metabolites of (I) which are peculiar to mated cultures of *B. trispora* is a series of neutral compounds, of which in our experience the major component is a substance which we have now characterized and designate as trisporol-C, (II). Isolated chromatographically, it has the molecular formula $C_{18}H_{28}O_3$ (from high resolution mass spectrometry); it has the characteristic trienone chromophore as seen in (I) and the fragmentation pattern in the mass spectrometer indicates the structure (II). This structure was confirmed by a partial synthesis from (Ia) by $LiAlH_4$ reduction of the corresponding ketal; compared with the natural material, the product had identical UV-, IR- and mass-spectra and was chromatographically indistinguishable on silica gel plates with a range of solvents and colour sprays. Since in the precursor-incorporation experiment reported below, the dilution of radioactivity in (II) was markedly less than in (Ia), we believe that this primary alcohol is a near precursor of the trisporic acids. Its physiological role is under investigation.

Earlier, by using 2-^{14}C-mevalonate we had shown that (I) contains the remains of 4 isoprene units, and adduced arguments that the monocyclic C_{18} skeleton of the trisporic acids arises from β-carotene[7]. In particular, when ^{14}C-β-carotene (labelled biosynthetically from 2-^{14}C-acetate)

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[1] Upjohn Co., Netherlands Patent No. 6,512,313 (1966).

[2] L. CAGLIOTTI, G. CAINELLI, B. CAMERINO, R. MONDELLI, A. PRIETO, A. QUILICO, T. SALVADORI and A. SELVA, Tetrahedron (Supplement) *7*, 175 (1966).

[3] D. M. THOMAS and T. W. GOODWIN, Phytochem. *6*, 355 (1967).

[4] D. M. THOMAS, R. C. HARRIS, J. T. O. KIRK and T. W. GOODWIN, Phytochem. *6*, 361 (1967).

[5] H. VAN DEN ENDE, J. Bact. *96*, 1298 (1968).

[6] D. J. AUSTIN, J. D. BU'LOCK and G. W. GOODAY, Nature *223*, 1178 (1969).

[7] D. J. AUSTIN, J. D. BU'LOCK and D. J. WINSTANLEY, Biochem. J. *113*, 34P (1969).

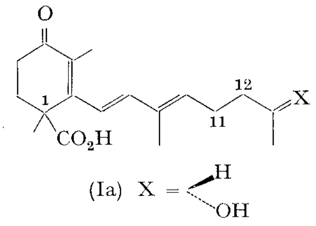
was fed to mated *B. trispora* the percentage incorporations and dilutions indicated that this was a more efficient precursor than was 2-^{14}C-mevalonate under comparable conditions.

Percent incorporation (and dilution factor) in:

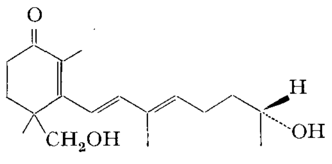
	(Ib)	(Ia)
from ^{14}C-β-carotene	2.5% (× 220)	0.9% (× 3,700)
from 2-^{14}C-mevalonate	0.03% (× 8,800)	0.6% (× 11,200)

Such data also suggest that the ketone (Ib) is a precursor of the alcohol (Ia).

Through the courtesy of the Hoffman-La Roche Company of Basle, a sample of 11,12-$^3H_2$-retinyl acetate was available. Retinal is known to occur in Phycomyces and seemed a possible intermediate between β-carotene and (II). The compound was fed to mated *B. trispora* and the isotope was very effectively incorporated into trisporol and the trisporic acids: in (II), incorporation 1.3%, dilution × 50; in (Ia), incoporation 1.9%, dilution × 450. The purified methyl ester from (Ia) was degraded by ozonolysis of the acetate followed by $LiAlH_4$ reduction[8], purifying the resultant diols as the *p*-nitrobenzoates. The propan-1,2-diol derivative [from $C_{(8)}$, $C_{(9)}$ and 9-Me of (Ia)] was virtually inactive and the (–)-4R-pentan-1,4-diol derivative[9], from $C_{(10)}$-$C_{(14)}$ of (Ia), contained 48% of the ^3H originally found in (Ia). After conversion of the pentandiol into 1-trityloxypentan-4-one and base-catalysed exchange in D_2O, 21% of the original ^3H [of (Ia)] was retained. It follows that in the original (Ia) at least 21% of the total ^3H was at $C_{(11)}$ (or, less plausibly, $C_{(10)}$) and at least 27% was at $C_{(12)}$ (or less plausibly, $C_{(13)}$ or $C_{(14)}$).



(Ib) X = :O



(II)

The nominal distribution of ^3H in the retinyl acetate which was fed is equal between $C_{(11)}$ and $C_{(12)}$, but since the synthesis includes *cis-trans* isomerisation of a double bond at $C_{(11)}$ the actual ^3H distribution may well have been unequal. An unequal distribution of ^3H between these 2 positions may alternatively have arisen during the biosynthesis of (Ia). Furthermore, in the degradation of (Ia), during $LiAlH_4$ reduction of the ozonide, $C_{(11)}$ is transiently α to aldehyde C=O and γ to an alkoxyl anion, and hence very liable to ^3H loss (as H_2). Thus, although some aspects of the data will only be clarified by more detailed experiments, our failure to account for some 52% of the ^3H present in (Ia) is explicable and does not affect the main conclusion that the retinyl acetate was converted into trisporic acids by a direct route. In the unperturbed system, we presume that retinal is the more probable intermediate and that this is formed by the normal breakdown of β-carotene.

The overall biosynthesis of the trisporic acids, and incidentally the 'positive feedback' nature of trisporate-mediated carotenogenesis, are therefore established. The mechanism now provides a clear chemical basis for understanding observations that, in mutants of Phycomyces with defective β-carotene synthesis, in which retinal is also absent[10], both 'gamone' production[11] and the resultant sexual differentiation[12] are impaired[13].

Zusammenfassung. Aus *Blakeslea trispora* wird eine neue Verbindung, Trisporol-C isoliert, ihre Struktur aufgeklärt und die Biosynthese der verwandten Trisporinsäuren aus β-Carotin und retinal untersucht.

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Manchester M13 9PL (England), 13 October 1969.

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[8] H. P. SIGG, Helv. Chim. Acta *47*, 1401 (1964).

[9] Establishes the *R*-configuration at $C_{(13)}$ of (Ia).

[10] G. MEISSNER and M. DELBRUCK, Plant Physiol. *43*, 1279 (1968).

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[12] M. HEISENBERG and E. CERDA-OLMEDO, Molec. gen. Genet. *102*, 187 (1968).

[13] The work is supported by grants from the Science Research Council (to D.J.A.) and Imperial Chemical Industries (to D.D.).

Characterization of By-Products of Fusicoccin in Culture Filtrates of *Fusicoccum amygdali* Del.

Fusicoccin is a highly active phytotoxic metabolite produced by submerged cultures of the phytopathogenic fungus *Fusicoccum amygdali* Del.[1]. Its structure (I) has been elucidated in these laboratories[2], and independently confirmed at Imperial College, London[3].

Thin-layer chromatography (silica gel G, 8% propan-2-ol in chloroform) of culture filtrates of *F. amygdali* consistently shows, besides fusicoccin, small amounts of 6 substances with lower Rf values. In what follows these are indicated as F II, F III, . . . F VII, in order of decreasing chromatographic mobility. 4 of them, namely F II, F III, F IV and F VII, are also formed when dilute solutions of fusicoccin are incubated at room temperature in buffer having the same pH (about 7.0) of the culture brew during the production phase, and probably arise non-enzymically from fusicoccin in the course of the fermentation[4]. Among