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THE STRUCTURE OF TRISPORIC-C ACID*†

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Abstract—Trisporic-C acid is the major component of a biologically active acidic fraction isolated from submerged cultures of *Choanephora trispora*. On the basis of the chemical and spectrochemical data and synthesis, structure I′—a monocyclic trienic hydroxyketo acid—has been assigned to the product. Trisporic-B acid differs from the C acid by the absence of an alcoholic hydroxyl group.

The chloroform extract of submerged cultures of two heterothallic strains of the phycomycete *Choanephora trispora* (Thaxter) Sinha,[3] a well known producer of β-carotene, yielded an acidic fraction endowed with remarkable biological activity. Addition of traces of this material to the cultures of the single strains of the fungus, considerably increased the yield of β-carotene.[4] Chemical investigation revealed that the acidic fraction, was a mixture of three substances (factors β_1, β_2 and β_3) named trisporic A, B and C acids. Trisporic-C acid constituted about 80% of the crude material, the B acid being about 15%, the A acid only 1—2%. The isolation of the three products in a pure state proved a difficult task owing to the instability of the free acids, as indicated by the rapid changes which occur in the UV spectra of freshly prepared solutions. The methyl esters are more stable and could be separated by chromatography on silica gel. The chemical and spectrochemical behaviour of these substances indicated their close relationship. The investigations were largely confined to the more abundantly available *trisporic-C-acid*.[5]

Trisporic-C acid is an oil with a peculiar smell; its *methyl ester* (I), prepared with diazomethane analysed for $C_{19}H_{28}O_4$, with $[\alpha]_D^{20°} -11°$ ($CHCl_3$). It contains a carboxylic function, an α,β-unsaturated ketonic system, an alcoholic hydroxyl and three conjugated double bonds including one conjugated to the ketonic carbonyl. The UV spectrum (CCl_4) of the methyl ester shows a maximum at 320 mμ (shoulders at 305 and 335 mμ) assignable to a strongly substituted conjugated trienone.

The IR spectrum of this ester contains bands at 3440 (OH), 1736 ($COOCH_3$), 1663 (conj. CO) and 1601 (C=C) cm^{-1}. The presence of a conjugated CO is confirmed

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* This work is dedicated to the memory of Professor H. Stephen.

† A preliminary account of this work appeared in part in *La Chimica e l'Industria* **46** 961 (1964).

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[3] Synonym: *Blakeslea trispora.*

[4] A. Prieto, C. Spalla, M. Bianchi and G. Biffi, communication at the *2nd International Fermentation Symposium.* London 13–17 April (1964).

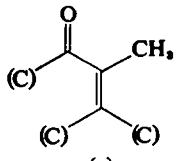
[5] To avoid repetitions, the same roman ordinal has been employed for the free acid and the corresponding methyl ester. Since in most cases the acids have been actually characterized in the form of their methyl esters, the free acid has been indicated when necessary.

by the reduction of I with $NaBH_4$ in methanol at room temperature; the IR spectrum of the product thus obtained does not contain the band at 1633 cm^{-1} and its UV spectrum (EtOH) shows maxima at 275, 285 and 296 mμ which are characteristic of a conjugated triene. Taking into account the functional groups and the double bonds, the molecular formula $C_{18}H_{26}O_4$ of trisporic-C acid indicates the presence of only one ring in its structure.

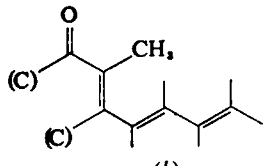
On account of the instability of trisporic-C acid the chemical investigation was performed on *tetrahydrotrisporic-C acid* (II′) obtained from I′ by hydrogenation under mild conditions. The UV spectrum of its *methyl ester* (II) shows a maximum at 249 mμ (EtOH) with log $\varepsilon = 4, 1$ in good agreement with the presence of an entirely substitued α,β-unsaturated ketone chromophore.[6] Its IR spectrum contains bands at 3460 (OH), 1735 ($COOCH_3$), 1664 (α,β-unsat. CO) and 1615 (C=C) cm^{-1}. Tetrahydrotrisporic-C acid methyl ester gives a *semicarbazone* (III) and a *monoacetate* (IV), in agreement with the presence of a carbonyl and an alcoholic hydroxyl group.

Further hydrogenation of II with Pd–C in ethanol affords the methyl ester (V), $C_{19}H_{34}O_4$, of the completely saturated *hexahydrotrisporic-C acid* (V′) as a mixture of two diastereoisomers which can partially be separated by chromatography on silica gel. The isolated single isomers are converted from one into the other and the original mixture restored simply by passing their solutions over alumina. Since the CO group has not been affected by hydrogenation, this fact can be interpreted as a process of equilibration on the carbon in α-position to the carbonyl, and confirms the presence of substituent on this carbon atom.

That the substituent is a methyl group is proved by the NMR spectra of II and of a number of its derivatives (see Table) which all contain a singlet of three protons at about 1·7 ppm. Concerning the environment of the CO chromophore in trisporic-C acid and its tetrahydroderivative, the following partial structure (*a*) which accounts for the formation of two diastereoisomers on hydrogenation of the third double bond, can further be developed to (*b*) if the presence of a conjugated triene system is taken into account.



(*a*)



(*b*)

The alcoholic hydroxyl group of tetrahydrotrisporic-C acid is secondary since with acetic anhydride in pyridine a *monoacetate* (IV) is formed, and cautious oxidation of II with chromic acid in acetone affords a diketo ester $C_{19}H_{30}O_4$ (VI). One of the two carbonyl groups of VI belongs to a methyl ketone as the NMR spectrum contains a signal of three protons at 2·05 ppm which is not present in the NMR spectrum of tetrahydrotrisporic-C acid ester (II), and is characteristic for a CH_3CO group. Further, the presence of a singlet of three protons centered at 1·40 ppm indicates that VI contains a methyl linked to a quaternary carbon atom, $CH_3C(C)_3$. Chemical evidence for the presence of a $—COCH_3$ in VI is supplied by the positive test with sodium nitroprusside and the results of drastic chromic acid oxidation of IXa and IXb.

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[*] This assignment is further supported by the NMR spectra of II and its derivatives which show no signal attributable to vinylic protons.

Table 1.
NMR Spectra*

Protons	II	VI	XII	XIII	VIIb	IXa	IXb	Xa	Xb	XIV
$COOCH_3$	3·6 s	3·67 s	—	—	3·58 s	—	—	—	—	3·58 s 3·68 s
CH_3CO	—	2·05 s	—	2·06 s	—	—	—	—	—	2·04 s
$CO-\underset{\underset{\mathbf{CH_3}}{\vert}}{C}=C$	1·7 s	1·74 s	1·70 d (J=1)	1·68 d (J=1)	—	—	—	—	—	—
$C-\overset{\overset{C}{\vert}}{\underset{\underset{\mathbf{CH_3}}{\vert}}{C}}-C$	1·4 s	1·40 s	—	—	1·17 s	1·02 s	0·90 s	1·03 s	0·95 s	1·28 s
$-\overset{\vert}{\underset{\underset{C\mathit{H}_3}{\vert}}{C}}H$	0·9 d (J≃6)	0·92 d (J≃6)	0·95 d (J≃6)	0·95 d (J≃6)	2CH$_3$ 0·88 d (J≃6)	†	†	†	†	0·84 d (J≃6)
$C=C-\underset{\underset{C\mathit{H}_3}{\vert}}{C}H$	—	—	1·19 d (J=7)	1·2 d (J=7)	—	—	—	—	—	—
$CH_3-\overset{\overset{C}{\vert}}{\underset{\underset{\mathit{H}}{\vert}}{C}}-O$	3·6 sextett (J=6)	—	3·68 sextett (J=6)	—	—	—	—	—	—	—
$C\mathit{H}_3-\overset{\overset{H}{\vert}}{\underset{\underset{C}{\vert}}{C}}-O$	1·1 d (J=6)	—	1·12 d (J=6)	—	—	—	—	—	—	—
$-OH$	2·6 s	—	2·5 s	—	—	2·0 s	—	—	—	—
$C-\overset{\overset{C}{\vert}}{\underset{\underset{C\mathit{H}_2OH}{\vert}}{C}}-C$	—	—	—	—	—	3·30 Quart AB (J=11)	3·5 Quart AB (J=11)	3·78 2H AB	4·00 Quart AB (J=11)	—
CH_3COO	—	—	—	—	—	—	—	1·97 s	2·00 s	—

* The NMR spectra were recorded in CCl_4 solution on Varian Associates A-60 spectrometer. The chemical shifts are expressed in ppm (δ) relatively to TMS as internal standard. The coupling constants (J) are given in c/s.

† Signals concealed by the overlapping of other absorptions.

Catalytic hydrogenation on Pd–C of diketo ester VI produces a mixture of two saturated *diketo esters* $C_{19}H_{32}O_4$ (VIIa and VIIb)[7] which can be separated by rapid chromatography on silica gel; the same mixture is obtained by cautious chromic acid oxidation of hexahydrotrisporic-C acid methyl esters (Va, b). Solutions of the pure diastereoisomers regenerate the original equilibrium mixture when passed over alumina. Wolff-Kishner reduction of VIIa + VIIb followed by esterification with diazomethane yields a mixture of two diastereoisomeric *di-deoxyhexahydrotrisporic-C acid methyl esters* $C_{19}H_{36}O_2$ (VIIIa + VIIIb), which are stable and can easily be separated by gas chromatography. Reduction with LAH of the mixture of the two esters (VIIIa + VIIIb) leads to a mixture in 1:1 ratio of two diastereoisomeric alcohols $C_{18}H_{36}O$ (IXa + IXb) which can be separated by chromatography over alumina. The NMR spectra of these substances show a quartet of signals of AB type (J = 11 c/s) at 3·3–3·5 ppm assignable to the two protons of a CH_2OH group linked to a quaternary

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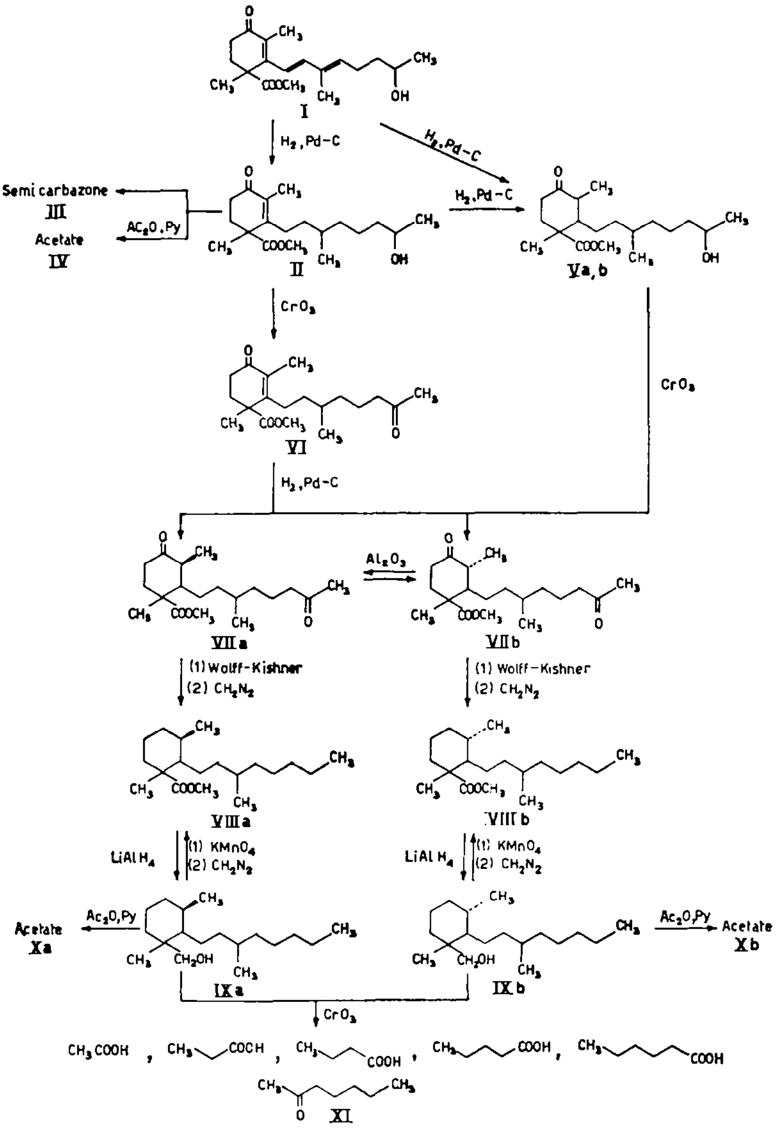
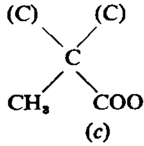
[7] The configurations indicated in Chart 1 for the diastereoisomers of (VIIa, VIIb); (IXa, IXb); (Xa, Xb), are purely arbitrary.

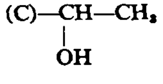
Chart 1



asymmetrical substituted carbon atom.[8] In the NMR spectra of the alcohols (IXa, IXb) and the *acetates* (Xa, Xb) there is a singlet of three protons at 0·92 ppm belonging to a methyl group carried by a quaternary carbon atom. These results, which are in agreement with those already reported for the diketo ester (VI), indicate that, besides (*b*) partial structures (*c*) and (*d*) are present in trisporic-C acid methyl ester (I):



(*c*)



$$(C)-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_3$$

(*d*)

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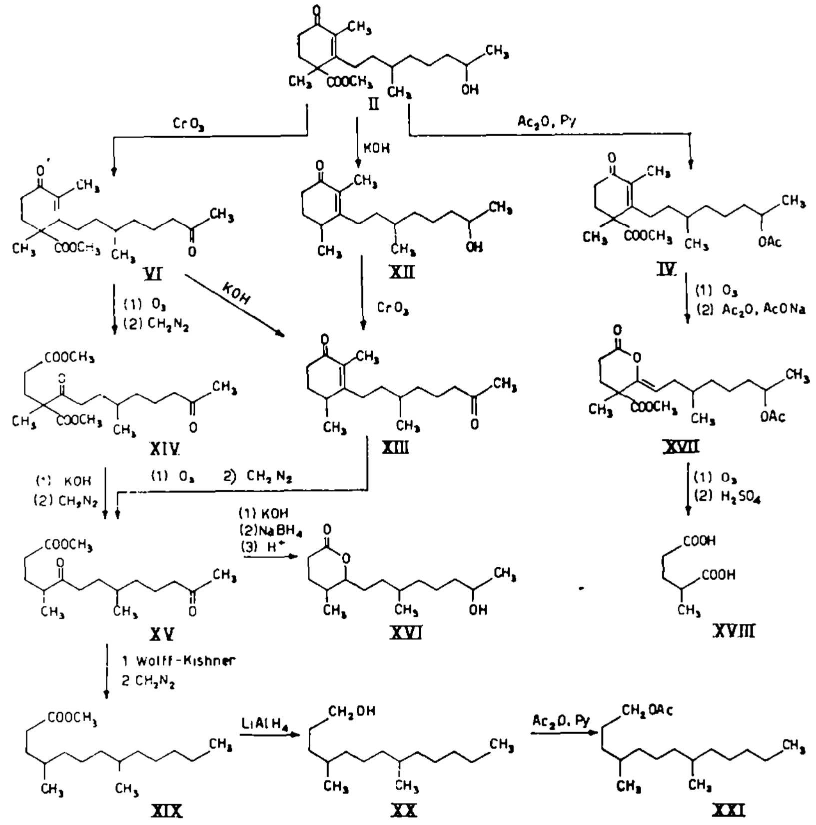
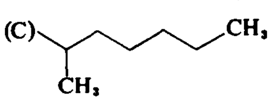
[8] In the corresponding acetates (Xa and Xb) these signals are shifted to 3·8 and 4·0 ppm.

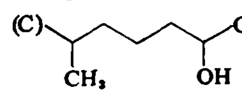
Chart 2



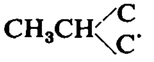
Valuable information concerning the non-cyclic moiety of trisporic-C acid has been supplied by the Karrer-Schmidt degradative oxidation of the alcohols (IXa and IXb) which affords in high yield a mixture of all the normal fatty acids C_2–C_5 and a volatile neutral product which has proved identical with *heptanone*-2 (XI). This fact demonstrates that the alcohols (IXa and IXb) contain an aliphatic chain C_7 of structure (*e*):



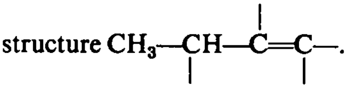
(*e*)

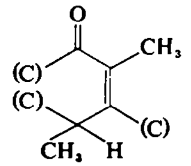


(*f*)

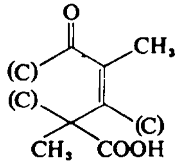
The presence of a methyl branch in the side chain of tetrahydrotrisporic-C acid is further confirmed by the NMR spectra of V, XIII and XI'V (see below) which all contain a doublet centered at 0·92–0·95 ppm with J = 6 c/s, assignable to a methyl  $CH_3CH\langle{}^{C}_{C}$. Chromic acid oxidation of the diketo ester (VI) yields acetic acid as the sole volatile acid, indicating that the second carbonyl group in VI and VIIa, b is adjacent to the terminal CH_3 of the side chain which in tetrahydrotrisporic-C acid (II') has therefore structure (*f*).

The action of boiling 15% methanolic KOH on diketo ester (VI) affords by loss of

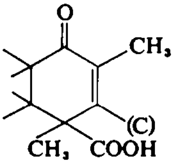
CO_2 a *diketone* $C_{17}H_{28}O_2$ (XIII) which still contains the α,β-unsaturated ketonic system and the terminal $COCH_3$. Its NMR spectrum shows a doublet centered at 1·2 ppm (J = 7 c/s) attributable to the methyl group of structure  $CH_3{-}\overset{|}{\underset{|}{C}}H{-}\overset{|}{C}{=}\underset{|}{C}{-}$. In the same spectrum the signal belonging to the CH_3 located on the C=C in α-position to the carbonyl, appears to be split (doublet centered at 1·68 ppm with J = 1 c/s) by interaction with a homoallylic proton, and this would suggest the presence in XIII of a partial structure (*g*)[9]. The same diketone (XIII) can be obtained from tetrahydrotrisporic-C acid methyl ester (II) by decarboxylation with boiling 15% methanolic KOH followed by cautious chromic acid oxidation of *keto alcohol* (XII). The carboxyl thus eliminated was not in β-position to the CO function but was situated at a β-vinylogous position as demonstrated not only by the NMR data given but also by the fact that similar basic treatment of a mixture of the two saturated diketoesters (VIIa and VIIb) which differ from VI only by the absence of the double bond conjugated to the CO, gives the corresponding acids without loss of CO_2. These results demonstrate the presence in tetrahydrotrisporic-C acid (II′) of the partial structure (*h*) which together with (*f*) accounts for the 18 carbon atoms of the trisporic-C acid skeleton:



(*g*)

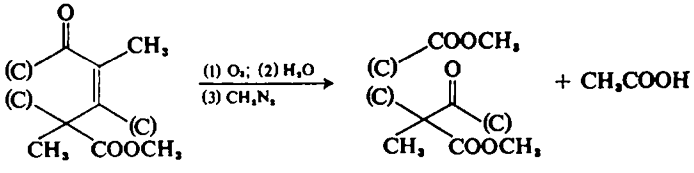


(*h*)



(*i*)

The presence of only one ring in the structure of trisporic-C acid and its derivatives is finally concluded from a study of the ozonolysis products of the diketo ester (VI) and the acetate of the ketol (IV). Ozonolysis at −70° in ethyl acetate solution of VI affords a *diketo di-acid*, characterized as its *dimethyl ester* $C_{18}H_{30}O_6$ (XIV) in accordance with the following scheme.



The molecular formula of XIV demonstrates that the two fragments are directly linked and that ozonolysis has resulted in the cleavage of a mono-unsaturated ring. Product XIV is a β-keto ester, and on boiling with 15% methanolic KOH loses one of the carboxyls with formation of a *monocarboxylic diketo acid* (methyl ester $C_{16}H_{28}O_4$, XV). Reduction with $NaBH_4$ of an aqueous solution of the sodium salt of diketo acid (XV′) followed by acidification of the alkaline solution, produces a neutral substance

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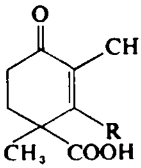
[9] The homoallylic coupling of the methyl group with the methylene of the side chain is practically non-existent because of the negligible value of ϑ; on the contrary, ϑ is about 90° for the ring methyl group. Compare with the NMR spectrum of VI in which the corresponding methyl appears as a singlet. See also J. H. Pinhey and S. Sterell, *Tetrahedron letters* 275 (1963).

[10] R. G. Curtis, I. Heilbron, E. R. H. Jones and G. F. Woods, *J. Chem. Soc.* 461 (1953).

$C_{15}H_{28}O_3$. Its IR spectrum shows bands at 3550 (OH) and 1735 (CO-lactone in six membered ring) cm^{-1}, and is consistent with structure XVI of the hydroxy-δ-lactone. This fact demonstrates that the carboxyl of XV′ is separated from one of the two CO groups by a chain of three carbon atoms; the α,β-unsaturated ketonic system originally present in VI must, therefore, belong to a C_6 ring, as shown in (*i*).

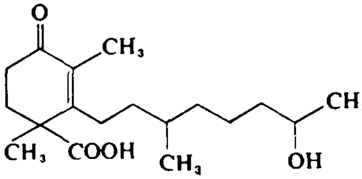
The complete structure elucidation of the cyclic moiety of tetrahydrotrisporic-C acid (II) has been achieved through the ozonolysis of the acetate (IV) followed by acetylation to the *acetate* $C_{19}H_{30}O_6$ (XVII) of an *enol-lactone* (its IR spectrum show bands at 1770 and 1680 cm^{-1} which are typical for a six-membered enolic lactone). Further ozonolysis of XVII, followed by decomposition with water and prolonged treatment with 30% sulphuric acid in order to decarboxylate the intermediate malonic acid, affords in good yield *α-methylglutaric acid* (XVIII). Significant amounts of α-methylglutaric acid have been obtained also by direct permanganate oxidation of trisporic-C acid (I′).

These results demonstrate that structure (k) can be assigned to tetrahydrotrisporic-C acid.



with R = $H_8C_{18}O$

(*k*)

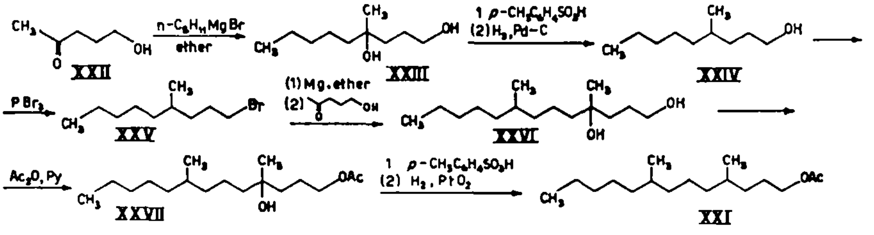


(*l*)

Taking into account what has been reported on the structure of side chain R, this formula can safely be developed to (*l*).

Further evidence of this structure has been obtained through the Wolff-Kishner reduction of diketo ester (XV) which gives a saturated monocarboxylic acid $C_{15}H_{30}O_2$. Mass spectrum measurements of its methyl ester $C_{16}H_{32}O_2$ in addition to confirming the mol wt (256), have demonstrated that the acid is branched at carbon atoms 4 and 8, and possesses the structure of *4,8-dimethyltridecanoic acid* (XIX′). Structure XIX has been confirmed by the synthesis of *4,8-dimethyltridecan-1-ol-acetate* (XXI), from pentan-1-ol-4-one, as shown in Chart 3.

Chart 3



The synthetic product was identical with the *acetate* of the *alcohol* (XX) obtained by LAH reduction of the *ester* (XIX). *Trisporic C acid* has therefore, structure I′ in which is still to be determined the stereochemistry of the double bonds and the two centres of asymmetry.

Investigations in progress on the structure of *trisporic-B acid* have demonstrated that it contains the same carbon skeleton as trisporic-C acid. In fact, catalytic hydrogenation on Pd-C followed by Wolff-Kishner reduction and esterification with diazomethane affords the same mixture of diastereoisometric esters (VIIIa and VIIIb) similarly obtained from trisporic-C acid. Trisporic-B acid does not contain the alcoholic hydroxyl group in the side chain, as shown by the IR spectrum of its methyl ester.

The biogenesis of trisporic-C and B acids is clearly via three isopentane units in the usual head to tail arrangement, and a C_3 residue derived by degradation of a C_5 unit.

EXPERIMENTAL

Separation and purification of trisporic-A, B and C acids

The chloroform extract of the culture liquids on treatment with $NaHCO_3$aq yielded the sodium salts (4 g/100 ml of the mixture) of the three acids. To 30 ml of this solution cooled at 0°, 20 ml 5% H_2SO_4 was added and the liberated acids immediately taken up in CH_2Cl_2. The organic layer was washed twice with 10 ml water, dried over anhydrous Na_2SO_4, and treated with a slight excess of diazomethane in CH_2Cl_2 for 2 min in the cold. The solution was then evaporated at room temp *in vacuo*, and the oily residue (about 1 g) dissolved in 30 ml CH_2Cl_2 and chromatographed over silica gel (Merck) under a N_2 atm. Elution with CH_2Cl_2 (500 ml) gave 20 mg of an oil devoid of selective absorption in the UV. Elution with CH_2Cl_2–acetone (99:1) yielded 15 mg of *trisporic-A acid methyl ester*, $[\alpha]_D^{20°}$ +19° (c = 0·8 in $CHCl_3$) which showed in the UV (CCl_4) a maximum at 300 mμ with shoulders at 304 and 334 m and in the IR bands at 1733, 1664 and 1629 cm^{-1}. Further elution with CH_2Cl_2–acetone 95:5 (500 ml) gave 150 mg of *trisporic-B acid methyl ester* (XXVIII), $[\alpha]_D^{20°}$ = +39° (c = 0·95, in $CHCl_3$). Finally, CH_2Cl_2–acetone 90:10 (500 ml) eluted 810 mg of *trisporic-C acid methyl ester* (I), $[\alpha]_D^{20°}$ = −11° (c = 1·0 in $CHCl_3$) which presented in the UV (CCl_4) a maximum at 320 mμ ($E_{1cm}^{1\%}$ = 710) with shoulders at 305 and 335 mμ and IR bands at 3436, 1736, 1664, 1603 cm^{-1}. The three compounds undergo considerable change on exposure to air and light.

Tetrahydrotrisporic-C acid methyl ester (II)

Owing the instability of free trisporic acids and their methyl esters, the hydrogenation was carried out on the sodium salts. The aqueous solution (50 ml) of the crude sodium salts (40 mg/ml, pH 7·0) was shaken under H_2 at 1 atm with 0·5 g 10% Pd–C. The reaction was allowed to proceed until the UV spectrum of the solution showed a maximum at 259 mμ (2 hr and 200 ml H_2). The catalyst was then filtered off and the solution acidified with dil H_2SO_4, and extracted with CH_2Cl_2. The organic layer was washed with water and dried over Na_2SO_4 and on evaporation left an oily residue (1·8 g) which was treated with excess diazomethane in CH_2Cl_2. The solvent was evaporated and the residue, dissolved in benzene–pet. ether (1:1) was passed through a column containing 56 g alumina (act. II). Benzene eluted 1·06 g of crude II. The product was purified by distillation *in vacuo* collecting the fraction b.p. 170–180° (0·2 mm). (Found: C, 70·21; H, 9·46. $C_{19}H_{30}O_4$ requires: C, 70·33; H, 9·94%.) The ester has $[\alpha]_D^{20°}$ = −37° ($CHCl_3$). Its UV spectrum (EtOH) shows a maximum at 249 mμ. The IR has bands at 3460, 1735, 1664 and 1615 cm^{-1}.

Semicarbazone (III). A solution of II (100 mg) in MeOH (10 ml) was treated with semicarbazide hydrochloride (100 mg) and pyridine (10 ml) and the mixture left overnight at room temp. After dilution with water, the semicarbazone was extracted with ether and the ethereal solution evaporated. Crystallization of the residue (110 mg) from aqueous MeOH gave crystals, m.p. 112–113° with $[\alpha]_D^{20°}$ −103° ($CHCl_3$). (Found: C, 62·97; H, 8·83; O, 16·70; N, 11·20. $C_{20}H_{35}O_4N_3$ requires: C, 62·92; H, 9·25; O, 16·78; N, 11·02%.)

Acetate (IV) *of tetrahydrotrisporic methyl ester* (II)

A solution of the ester (500 mg) in acetic anhydride (5 ml) and pyridine (5 ml) was heated on a steam bath for 2 hr. The reaction mixture was poured into water and extracted with ether. The ethereal layer, washed with water and dried over Na_2SO_4, furnished after removal of the solvent an oil which was chromatographed on a column of alumina (15 g, act. II). By elution with benzene

400 mg of IV were obtained, b.p. 180–190° (0·2 mm). (Found: C, 69·11; H, 9·58. $C_{21}H_{34}O_5$ requires: C, 68·82; H, 9·35%), UV λ_{max} 249 mμ (log $\varepsilon = 4{\cdot}1$); IR (liq.) bands at 1742, 1681 and 1618 cm^{-1}.

Hexahydrotrisporic-C acid methyl ester (Va, b)

An aqueous solution (pH 7·0) of the crude sodium salts of trisporic acids (100 ml, containing about 4 g of acids) was shaken under H_2 at 2 atm with 5 g 10% Pd–C. The reaction was allowed to proceed for 24 hr, until the complete disappearance of the maximum at 259 mμ in the UV spectrum of the solution. After removal of the catalyst the solution was acidified with 2N H_2SO_4, and extracted 3 times with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4 and on evaporation left a yellowish oil (3·5 g) which was treated with excess diazomethane in CH_2Cl_2 solution. The solvent was evaporated and the residue distilled *in vacuo* collecting the fraction b.p. 170–180° (0·2 mm). (Found: C, 69·76; H, 10·32. $C_{19}H_{34}O_4$ requires: C, 69·90; H, 10·50%.)

Chromic acid oxidation of tetrahydrotrisporic-C acid methyl ester (II) *to diketo ester* (VI)

A solution of II (1 g) in acetone (50 ml) was treated at room temp with Kiliani reagent[10] (2 ml). After 5 min MeOH (5 ml) was added, the solvent removed *in vacuo*, and the residue extracted with ether. The ethereal solution was washed with water, dried over Na_2SO_4 and on evaporation, gave 950 mg oily diketo ester (VI), b.p. 150–154° (0·2 mm), $[\alpha]_D^{20°}$ −69° ($CHCl_3$), UV (EtOH) λ_{max} 249 mμ (log $\varepsilon = 4{\cdot}0$); IR (liq) bands at 1730, 1653 and 1613 cm^{-1}.

Chromic acid oxidation of hexahydrotrisporic-C acid methyl ester (Va, b) *to the saturated diketo ester* (VIIa + VIIb)

The crude methyl ester (Va, b) prepared as described from 100 ml of original solution of sodium salts, was dissolved in 100 ml acetone and treated at room temp with 7 ml Kiliani reagent. After 8 min, 10 ml MeOH was added, the solvents evaporated *in vacuo*, and the residue extracted with ether. The ethereal solution was washed with water, dried over Na_2SO_4, and evaporated. The oily residue (3·1 g) dissolved in pet. ether was passed through a column containing 60 g of alumina (act. II). Benzene eluted 2·05 g diketo ester. The product (TLC) appeared to be a mixture of two diastereoisomers (VIIa and VIIb), IR (liq), band at 1733 cm^{-1}.

Hydrogenation of diketo ester (VI) *to the saturated diketo esters* (VIIa + VIIb)

A solution of VI (900 mg) in EtOH (50 ml) was shaken at room temp under H_2 with 500 mg 10% Pd–C. The reaction was allowed to proceed until complete disappearance of the peak at 249 mμ in the UV spectrum. The catalyst was filtered off and the filtrate evaporated *in vacuo*. The oily residue was identical with the mixture of VIIa and VIIb described (TLC and IR spectrum).

Separation of VIIa *and its interconvertion into the equilibrium mixture* VIIa + VIIb

A solution of the mixture (1 g) of the diastereoisomeric diketo esters (VIIa + VIIb) in pet. ether was passed through a column charged with 70 g neutral silica. Pet. ether–benzene (1:1) eluted a fraction (50 mg) which proved (TLC) to be the pure less polar diastereoisomer (VIIa). This compound was dissolved in pet. ether and passed through a column containing 1 g of alumina (activity II) and benzene eluted 40 mg of the mixture (VIIa + VIIb).

Wolff-Kishner reduction of diketo esters (VIIa *and* VIIb)

Di-deoxyhexahydrotrisporic-C acid methyl esters (VIIIa *and* VIIIb). A solution of the mixture (VIIa + VIIb; 3 g) in ethylene glycol (15 ml), 99% hydrazine hydrate (5 ml) and EtOH (5 ml) was heated under reflux for 2 hr. Solid NaOH (3 g) was then added and the solvents distilled off until the temp of the boiling solution reached 180°. After 4 hr heating under reflux the reaction mixture was cooled and poured into excess 2N H_2SO_4. The acidic solution was extracted with ether, the ethereal layer washed with water, dried over Na_2SO_4 and evaporated. The oily residue (2·2 g) was treated with excess diazomethane in CH_2Cl_2 and allowed to react for 15 min. Evaporation *in vacuo* of the solvent gave a residue which was dissolved in hexane and passed through a column filled with 60 g alumina (act. II). Hexane eluted 1·5 g of a mixture of two diastereoisomeric di-deoxyhexahydrotrisporic esters (*Rf* very similar), which were separated by gas chromatography. The product VIIIa was more polar on TLC: b.p. 115° (0·2 mm), $[\alpha]_D^{20°} = +12°$ ($CHCl_3$). (Found: C, 76·85;

H, 12·32. $C_{19}H_{36}O_2$ requires: C, 76·97; H, 12·24%); IR (liq) band at 1736 cm^{-1}. The product VIIIb was less polar on TLC: b.p. 115° (0·2 mm), $[\alpha]_D^{20°} = +11°$ ($CHCl_3$). (Found: C, 77·16; H, 12·18. $C_{19}H_{36}O_2$ requires: C, 76·97; H, 12·24); IR (liq), band at 1736 cm^{-1}.

Reduction with lithium aluminium hydride of esters (VIIIa *and* VIIIb) *to alcohols* (IXa *and* IXb)

To a solution of VIIIa (100 mg) in dry ether (20 ml) LAH (100 mg) was added, and the mixture heated under reflux on a water bath for 2 hr. The excess hydride was then decomposed with water and the mixture extracted with ether. The ethereal layer was washed with dil H_2SO_4 and water, dried over Na_2SO_4 and evaporated, yielding oily IXa (95 mg), b.p. 120° (0·2 mm), $[\alpha]_D^{20°} = -7°$ ($CHCl_3$). (Found: C, 80·26; H, 13·47; O, 6·34. $C_{18}H_{36}O$ requires: C, 80·52; H, 13·52; O, 5·96%); IR (liq) band at 3356 cm^{-1}.

Similar treatment of VIIIb gave IXb, b.p. 120° (0·2 mm), $[\alpha]_D^{20°} = +1°$ ($CHCl_3$). (Found: C, 80·37; H, 13·47; O, 6·57. $C_{18}H_{36}O$ requires: C, 80·52; H, 13·52; O, 5·96%); IR (liq) band at 3356 cm^{-1}.

Acetates Xa *and* Xb *of* IXa *and* IXb. The alcohol IXa (100 mg) was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) on a steam bath for 2 hr. The mixture was then treated with water and the mixture worked up with ether. The ethereal layer washed with water and dried over Na_2SO_4 left after evaporation of the solvent 95 mg crude acetate which was purified by chromatography on alumina (act. II, eluent hexane) yielding 90 mg of acetate (Xa), b.p. 110° (0·2 mm), $[\alpha]_D^{20°} = -9·5°$ ($CHCl_3$). (Found: C, 77·58; H, 12·29. $C_{20}H_{38}O_2$ requires: C, 77·36; H, 12·34%); IR (liq.) band at 1748 cm^{-1}. In a similar way acetate (Xb), b.p. 112° (0·2 mm), $[\alpha]_D^{20°} = +6°$ ($CHCl_3$) was obtained. (Found: C, 77·50; H, 12·28; O, 10·34. $C_{20}H_{38}O_2$ requires: C, 77·36; H, 12·34; O, 10·31%); IR (liq.) band at 1748 cm^{-1}.

The acetates (Xa and Xb) were converted to the corresponding alcohols (IXa and IXb) on treatment for 2 hr with LAH in ether heated under reflux.

Conversion of alcohols (IXa *and* IXb) *into methyl esters* (VIIIa *and* VIIIb)

To a stirred solution of IXa (50 ml) in acetone (10 ml), powdered $KMnO_4$ (50 ml) was added, and the reaction mixture allowed to stand 5 hr at room temp. A few drops of MeOH were added to destroy the excess of oxidant and the mixture acidified with dil H_2SO_4, was extracted with ether. The ethereal layer was washed with water, dried over Na_2SO_4, and evaporated. The residue (40 mg) was treated for 10 min with an excess diazomethane in CH_2Cl_2 and on distillation of the solvent 35 mg of VIIIa were obtained. In a similar way IXb was converted into VIIIb.

Karrer-Schmidt oxidation of alcohols (IXa *and* IXb) *and of diketo ester* (VI)

The alcohol IXa (150 mg) was oxidized with boiling Karrer-Schmidt chromic acid solution (40 ml) in a Claisen flask equipped with descending condenser and dropping funnel. After about 90 min, 60 ml of liquid were distilled off and immediately replaced by water from the funnel. The distillate was neutralized with N/10 NaOH (9·8 ml) and again distilled. The condensate contained a neutral product which, extracted with ether and submitted to gas chromatography, proved to be identical with *2-heptanone* (XI). The residual solution was acidified with 2N H_2SO_4 and the liberated acids extracted with ether. Gas chromatography of the ethereal extract dried over Na_2SO_4 proved the presence of the following normal fatty acids: acetic and valeric (both abundant), propionic and caproic (traces). Identical results were obtained in the oxidation of the alcohol IXb.

The diketo ester VI (150 mg) was oxidized under similar conditions but acetic acid was the only acidic volatile fraction.

Decarboxylation of diketo acid (VI′) *to diketone* (XIII)

The diketo ester VI (400 mg) was refluxed for 4 hr with 15% methanolic KOH (50 ml) under N_2 atm. Water was then added and the alkaline mixture extracted with ether. The ethereal layer, washed with water and dried over Na_2SO_4 left after evaporation of the solvent 200 mg of crude XIII. By chromatography over alumina (6 g, act. II), hexane–benzene (1:1), eluted 130 mg pure XIII. The product was identical with a sample of XIII prepared from II as reported below.

Decarboxylation of tetrahydrotrisporic-C acid (II′) *to ketoalcohol* (XII)

Compound II (1 g) was heated under reflux (3 hr) under an N_2 atm with 15% methanolic KOH (50 ml). The alkaline mixture, diluted with water, was extracted with ether; 480 mg of a neutral compound were obtained and were passed through a column containing 15 g of alumina (act. II). Benzene–ether (9:1) eluted 415 mg of XII, b.p. 220° (0·5 mm), $[\alpha]_D^{20^\circ} = 0^\circ$ ($CHCl_3$). (Found: C, 76·48; H, 11·13. $C_{17}H_{30}O_2$ requires: C, 76·64; H, 11·35%); UV, $\lambda_{max}^{EtOH} = 248$ mμ (log ε = 4·1); IR (liq) bands at 3472, 1675, 1618 cm^{-1}.

Chromic acid oxidation of ketoalcohol (XII) *to diketone* (XIII)

A solution of XII (60 mg) in acetone (10 ml) was treated for 5 min at room temp with 0·1 ml Kiliani reagent, and then MeOH (3 ml) was added and the reaction mixture evaporated *in vacuo*. The residue was extracted with ether and the ethereal layer yielded 55 mg of oily diketone (XIII), b.p. 210° (0·5 mm), $[\alpha]_D^{20^\circ} = +11^\circ$. (Found: C, 76·90; H, 10·58. $C_{17}H_{28}O_2$ requires: C, 77·22; H, 10·67%); UV $\lambda_{max}^{EtOH} = 248$ mμ (log ε = 4·15); IR (liq) bands at 1712, 1668, 1613 cm^{-1}.

Ozonolysis of diketo ester (VI): *diketo di-acid ester* (XIV)

A solution of VI (450 mg) in AcOEt (20 ml) was submitted for 25 min at −55° to a slow stream of ozonized O_2. Most of the solvent was then removed *in vacuo* (caution) and the resulting syrup refluxed for 5 hr with 50 ml water. Ether was added to the reaction mixture and the ethereal layer extracted with 100 ml 5% K_2CO_3aq. The alkaline solution was acidified with dil H_2SO_4 and extracted with ether. The organic layer, washed with water and dried over Na_2SO_4, left after removal of the solvent an oily residue (400 mg) which was treated with excess diazomethane in CH_2Cl_2. After 15 min the solution was evaporated *in vacuo* and the residue chromatographed on 12 g fluorisil. Hexane–benzene (1:1) eluted 350 mg of XIV, b.p. 190° (0·5 mm), $[\alpha]_D^{20^\circ}$ −7·4° ($CHCl_3$). (Found: C, 63·11; H, 8·78. $C_{18}H_{30}O_6$ requires: C, 63·13; H, 8·83%); IR (liq.) bands at 1745 and 1712 cm^{-1}.

Decarboxylation of diketo di-acid (XIV′) *to diketo mono-acid* (XV′)

Compound XIV (100 mg) was refluxed for 2 hr under N_2 atm with 20 ml of 5% KOH (in MeOH–water 1:1). After dilution with water the reaction mixture was repeatedly washed with ether, and the aqueous layer, acidified with dil H_2SO_4, was extracted with ether. The ethereal layer, dried over Na_2SO_4, afforded after elimination of the solvent an oily residue (90 mg) which was treated with excess diazomethane in CH_2Cl_2. The residue obtained after removal of the solvent *in vacuo* was chromatographed over fluorisil (3 g). Hexane–benzene (3:1) eluted 74 mg of oily XV, b.p. 160° (0·5 mm), $[\alpha]_D^{20^\circ} = +2^\circ$ ($CHCl_3$). (Found: C, 67·38; H, 9·83. $C_{16}H_{28}O_4$ requires: C, 67·57; H, 9·93%); IR (liq.) bands at 1739 and 1712 cm^{-1}.

Ozonolysis of diketone (XIII): *diketo ester* (XV)

A solution of XIII (1·5 g) in AcOEt (100 ml) was submitted for 30 min at −76° to a slow stream of ozonized O_2. Most of the solvent was cautiously removed *in vacuo*, and the residual syrup refluxed with 100 ml water for 5 hr. The mixture was extracted with ether, and the organic layer shaken with 500 ml 5% K_2CO_3aq. The alkaline solution was acidified with dil H_2SO_4 and extracted with ether. The ethereal layer, washed with water and dried over Na_2SO_4, left after removal of the solvent an oily residue which was treated with excess diazomethane in CH_2Cl_2. The solvent was evaporated and the residue (1·1 g) was passed through a column filled with 35 g fluorisil. Hexane–benzene (3:1) eluted 960 mg of XV b.p. 160° (0·5 mm). (Found: C, 67·68; H, 9·64. $C_{16}H_{28}O_4$ requires: C, 67·57; H, 9·93%.) The product was identical with XV obtained from XIV as previously reported.

Reduction with sodium borohydride of diketo monoester (XV): *lactone* (XVI)

Compound XV (200 mg) was refluxed for 2 hr with 7% KOHaq (15 ml). The alkaline solution was acidified with 2N H_2SO_4 and extracted with ether; the organic layer was evaporated and the residue dissolved in 1N NaOH. To this solution, heated to 90°, small portions of $NaBH_4$ (200 mg) were added. After 1 hr the reaction mixture was cooled, acidified with dil H_2SO_4 and extracted with ether. The ethereal layer was washed with water, dried over Na_2SO_4 and evaporated. The oily residue was passed through a column containing alumina (6 g, act. II) and benzene eluted XVI (150 mg), b.p. 260° (0·5 mm), $[\alpha]_D^{20^\circ} = 0^\circ$. (Found: C, 69·45; H, 10·97. $C_{15}H_{28}O_3$ requires: C, 70·27; H, 11·01%); IR (liq.) bands at 3521 and 1736 cm^{-1}.

Ozonolysis of the acetate (IV) *of tetrahydrotrisporic-C acid methyl ester and subsequent treatment with acetic anhydride*

Lactone (XVII). A solution of IV (1 g) in AcOEt (40 ml) was submitted for 30 min at −65° to a stream of ozonized O_2. After cautious removal of the solvent *in vacuo* the resulting syrup was refluxed for 5 hr with 50 ml water. The reaction mixture was shaken with ether and the ethereal layer extracted with 200 ml 5% K_2CO_3aq. The alkaline solution was then acidified and extracted with ether. The oily residue obtained after removal of the solvent was refluxed for 3 hr with acetic anhydride (30 ml) and anhydrous sodium acetate (3 g). From the reaction mixture by evaporation *in vacuo*, treatment of the residue with water and extraction with ether, 700 mg of crude lactone (XVII) were isolated. Chromatography over alumina (25 g, act. II) and elution with benzene, afforded 600 mg of XVII, b.p. 230° (0·5 mm), $[\alpha]_D^{20°} = -5°$ ($CHCl_3$). (Found: C, 63·72; H, 8·34. $C_{19}H_{30}O_6$ requires: C, 64·38; H, 8·53%); IR (liq.) bands at 1770, 1736 and 1681 cm^{-1}.

Ozonolysis of lactone (XVII)

α-Methylglutaric acid (XVIII). A solution of XVII (200 mg) in AcOEt (20 ml) was submitted for 25 min at −78° to a stream of ozonized O_2. After cautious removal of the solvent *in vacuo* 50 ml of water was added and the mixture refluxed for 5 hr. The organic layer obtained by extraction with ether was shaken with 5% K_2CO_3aq and the alkaline solution, acidified with 2N H_2SO_4 X, again extracted with ether. Elimination of the solvent gave a residue which was refluxed for 8 hr with 30% H_2SO_4aq. The acidic solution was extracted 3 times with ether, and the ethereal layer dried and evaporated. On treatment of the residue with excess diazomethane in CH_2Cl_2 and removal of the solvent an oily ester was obtained which proved to be identical (gas chromatography and IR spectrum) with an authentic sample of α-methylglutaric acid dimethyl ester.

Wolff-Kishner reduction of diketo ester (XV) *to* 4,8-*dimethyltridecanoic acid* (XIX′)

Compound XV (300 mg) was refluxed for 2 hr with hydrazine hydrate (5 ml), EtOH (5 ml) and ethylene glycol (15 ml). Solid NaOH (2 g) was then added and the solvents distilled off until the boiling solution reached the temp of 180°. After 4 hr refluxing, the reaction mixture was cooled and treated with excess 2N H_2SO_4. Extraction with ether followed by elimination of the solvent gave an oily residue (290 mg) which was treated with excess diazomethane in CH_2Cl_2. After 15 min, the solvent was removed and the residue passed through a column of alumina (act. II; 15 g). Hexane eluted 250 mg of XIX, b.p. 130° (0·5 mm), $[\alpha]_D^{20°} = +2°$ ($CHCl_3$). (Found: C, 74·98; H, 12·47. $C_{16}H_{32}O_2$ requires: C, 74·94; H, 12·58%); Mol. wt (mass spectrography): Found 256. Calc. for $C_{16}H_{32}O_2$: 256.

Acetate (XXI) *of* 4,8-*dimethyltridecan*-1-*ol* (XX)

To a solution of XIX (100 mg) in dry ether (20 ml), LAH (200 ml) was added and the mixture refluxed for 2 hr. After decomposition of excess hydride with water, the ethereal layer was separated, washed with 10% H_2SO_4aq (10 ml) and water, dried over Na_2SO_4 and evaporated. The residue (80 mg) was treated with a mixture of acetic anhydride (0·5 ml) and pyridine (1 ml) and left at room temp for 2 hr. Water was then added (10 ml) and the acetate extracted with ether. From the ethereal layer, washed successively with dil H_2SO_4, water, 5% $NaHCO_3$aq and again water, 80 mg of oily acetate (XXI) were obtained, b.p. 100–110° (0·2 mm), IR (liq.) band at 3420 (OH) cm^{-1}.

Synthesis of the acetate (XXI) *of* 4,8-*dimethyltridecan*-1-*ol* (XX) (See Chart 3)

To the ethereal solution of n-amylmagnesium bromide prepared from n-amylbromide (45 g), ether (100 ml) and Mg turnings (7·2 g), XXII (10 g) was added and the mixture refluxed for 2 hr on a steam bath. The reaction mixture was then cooled, decomposed with NH_4Claq (400 ml), and extracted with ether. The organic layer, washed with water and dried over Na_2SO_4, furnished after removal of the solvent 20 g of crude 4-*methylnonan*-1,4-*diol* (XXIII). Without further purification 50 mg of *p*-toluensulphonic acid were added to the product and the mixture slowly distilled at ordinary press. The fraction (14 g) b.p. 210–230° (760 mm) was dissolved in EtOH (100 ml) and hydrogenated at room temp in the presence of 500 mg 10% Pd–C. The solution, filtered from the catalyst and evaporated, gave 12 g of an oily residue. The crude 4-*methylnonan*-1-*ol* (XXIV) thus obtained was purified by distillation (collecting the fraction b.p. 110–135° (0·2 mm) and successive chromatography in a column of alumina (act. II; 360 g). Benzene eluted 8 g of the pure alcohol, b.p. 210–220°.

(Found: C, 75·65; H, 14·09. $C_{10}H_{22}O$ requires: C, 75·88; H, 14·01%); IR (liq.) band at 3450 (OH) cm^{-1}. Compound XXIV (7 g) dissolved in dry hexane (20 ml) was treated at −15° with 1·5 g (10% excess) PBr_3. The mixture was allowed to reach 25° and kept at this temp overnight. The reaction mixture was then poured into a saturated ice-cold $NaHCO_3$aq (200 ml) and extracted with ether. The ethereal layer, washed with water and dried over Na_2SO_4, left after removal of the solvent 7·5 g of an oily residue. The crude XXV thus obtained was distilled *in vacuo* collecting the fraction, b.p. 100–105° (14 mm) and further purified by chromatography over alumina (22 g, act. II). Hexane eluted 3·2 g pure bromide, b.p. 100° (6 mm). (Found: C, 53·11; H, 9·12. $C_{10}H_{21}Br$ requires: C, 54·35; H, 9·57%.) Its IR spectrum (liq.) showed no band for an OH group. To a solution of XXV (2·8 g) in 100 ml dry ether, Mg turnings (310 ml) were added and the mixture refluxed for 2 hr under N_2 atm. After cooling, XXII (640 ml) dissolved in ether (20 ml) were introduced, and the mixture refluxed for 5 hr in a N_2 atm. Decomposition of the cooled reaction mixture with 50 ml 10% NH_4Claq and evaporation of the ethereal layer afforded 1·5 g crude 4,8-*dimethyltridecan*-1,4-*diol* (XXVI) which was further purified by chromatography on silica (45 g) yielding 1·2 g of the pure diol.

Acetylation of 1·1 g of the diol at room temp with 3 ml acetic anhydride and 5 ml pyridine gave 1·1 g of 4,8-*dimethyltridecan*-1,4-*diol*-1-*acetate* (XXVII), IR (liq.) band at 3460 (OH) and 1730 (acetyl) cm^{-1}.

The crude acetate (1 g) was dehydrated to the corresponding unsaturated alcohol acetate by distillation (0·2 mm) with 15 mg *p*-toluensulphonic acid. The distillate, dissolved in 20 ml MeOH, was shaken with H_2 in the presence of 100 mg of Adams PtO_2. The solution was filtered from the catalyst, water was added, and the mixture extracted with ether. From the ethereal layer, 0·5 g of crude 4,8-*dimethyltridecanol*-1-*acetate* (XXI) were isolated and purified by chromatography over 15 g of alumina (act. II). The product, b.p. 100–115 (0·2 mm) was identical (IR, TLC) with the acetate obtained from XIX.

Di-deoxyhexahydrotrisporic-C acids methyl esters (VIIIa *and* VIIIb) *from trisporic-B acid* (XXVIII)

A solution of trisporic-B acid methyl ester (100 mg) in EtOH (20 ml) was shaken with H_2 at 1 atm and 50 mg 10% Pd–C, and the reaction was allowed to proceed until the adsorption of H_2 had practically ceased. The solution was then filtered from the catalyst and evaporated. The residue was submitted to Wolff-Kishner reduction as described for the reduction of VIIa and VIIb to VIIIa and VIIIb. The acidic compound obtained was esterified with diazomethane in CH_2Cl_2 solution. IR spectra and gas chromatography data showed that the product was a mixture of the esters VIIIa and VIIIb.

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