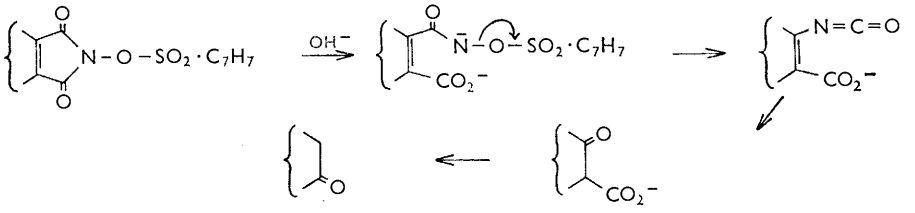
332. *The Nonadrides. Part IV.*[1,2] *The Constitution and Stereochemistry of Byssochlamic Acid*

By J. E. Baldwin, D. H. R. Barton, and J. K. Sutherland

Experiments are described which confirm the constitution of byssochlamic acid as determined by *X*-ray crystallography on a suitable heavy-atom derivative. The absolute configuration of byssochlamic acid has been determined by fission of the nine-membered ring to give acidic fragments of known absolute configurations. Various transformations of byssochlamic acid are reported which can be understood in terms of the established constitution. Photobyssochlamic acid has been obtained by irradiation of byssochlamic acid.

The constitution and relative stereochemistry of byssochlamic acid have been defined as (I) by *X*-ray investigations on the derived bis-*p*-bromophenylhydrazide (II).[3] We now report our chemical studies on the constitution and absolute stereochemistry of this interesting mould metabolite.

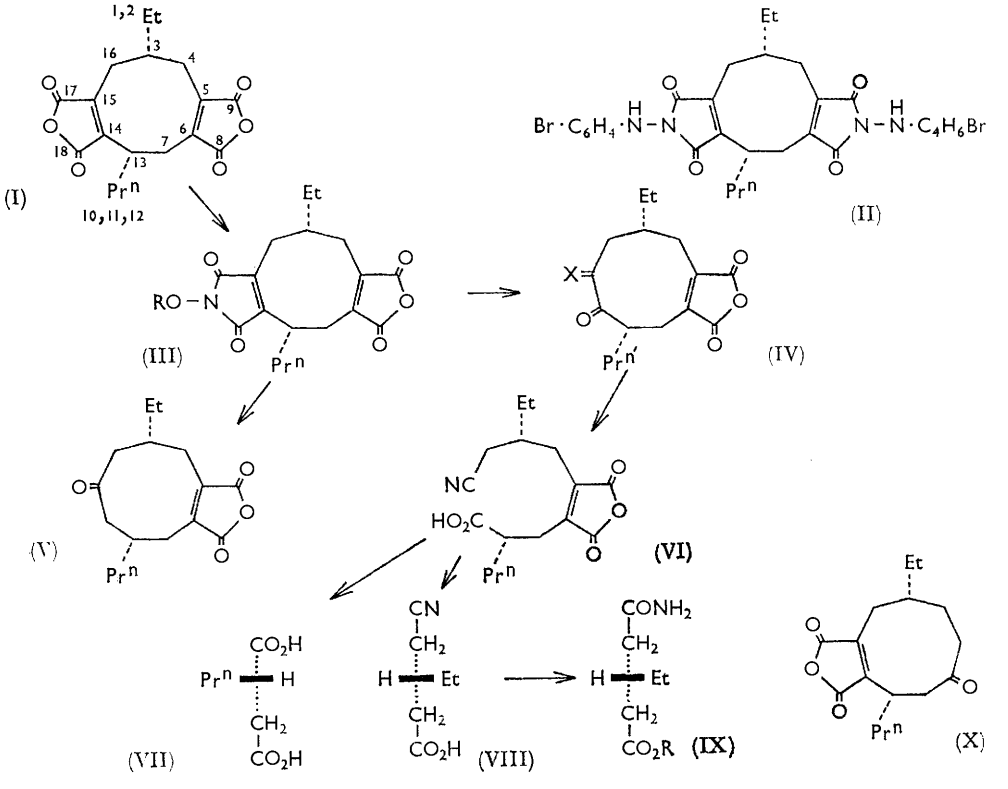
In agreement with previous work [4] we have found that the ethylenic linkages in byssochlamic acid are extremely inert. In order to degrade the molecule it was, therefore, necessary to modify the anhydride linkages in some way. We developed a modified Lossen rearrangement [5] as follows. With one mol. of hydroxylamine hydrochloride, byssochlamic acid tetrasodium salt gave a non-crystalline derivative (III; R = H). The presence of an anhydride ring was shown by infrared bands whilst the *N*-hydroxy-imide function could be detected by bands at 1800, 1720, and 3300 cm.$^{-1}$, in agreement with the data reported for *N*-hydroxymaleimide.[6] Treatment of the *N*-hydroxy-imide with toluene-*p*-sulphonyl chloride gave the derivative (III; R = $SO_2 \cdot C_7H_7$), which was hydrolysed with N-sodium hydroxide at 90° to a mixture of the ketones (IV; X = H_2) and (V), which were formed in 26% and 11% overall yield, respectively, from (I). We presume that ketone formation can be understood according to the following general scheme.



The ketone (IV, X = H_2) reacted with pentyl nitrite and hydrogen chloride in ether [7] to afford the α-hydroxyimino-ketone (IV; X = N·OH). When shaken with toluene-*p*-sulphonyl chloride and dilute alkali this underwent a second-order Beckmann transformation to furnish the nitrile-acid (VI), characterised as its methyl ester. Now that the medium ring had been opened the ethylenic linkage in the anhydride ring was more readily attacked. Ozonolysis in ethyl acetate followed by oxidation with alkaline hydrogen peroxide gave a mixture of acids separated by chromatography into (*S*)(−)-n-propylsuccinic acid (VII) [8] and the liquid half-nitrile of β-ethylglutaric acid (VIII), converted into β-ethylglutaric acid on vigorous acid hydrolysis. The ozonolysis was repeated and the

[1] Part III, D. H. R. Barton, L. D. S. Godinho, and J. K. Sutherland, preceding Paper.
[2] Preliminary communication, J. E. Baldwin, D. H. R. Barton, J. L. Bloomer, L. M. Jackman, L. Rodriguez-Hahn, and J. K. Sutherland, *Experientia*, 1962, **18**, 345.
[3] I. C. Paul, G. A. Sim, T. A. Hamor, and J. M. Robertson, *J.*, 1963, 5505.
[4] J. N. Ashley, P. Clutterbuck, and H. Raistrick, unpublished observations.
[5] See E. C. Franklin, *Chem. Rev.*, 1934, **14**, 219.
[6] D. E. Ames and T. F. Grey, *J.*, 1955, 631, 3518.
[7] J. A. Barltrop, A. J. Johnson, and G. D. Meakins, *J.*, 1951, 181.
[8] K. Freudenberg and W. Lwowski, *Annalen*, 1955, **594**, 76.

reaction product treated more vigorously with alkaline hydrogen peroxide. Conversion of the acidic products into their *p*-phenylphenacyl esters gave the bis-ester of (*S*)(−)-n-propylsuccinic acid together with the *p*-phenylphenacyl ester (IX; R = CH_2·CO·C_6H_4Ph). An authentic sample of the racemic acid (IX; R = H) was prepared by reaction of the anhydride of β-ethylglutaric acid with ammonia, but in our hands this half-amide could not be resolved. As an alternative, the nitrile-acid (VIII), prepared from the ester (IX; R = Me) by treatment with toluene-*p*-sulphonyl chloride in pyridine,[9] followed by careful hydrolysis, was resolved through its quinine metho-salt, to furnish the pure (+)-isomer of the acid (VIII). Reaction of the latter with alkaline hydrogen peroxide afforded the (*R*)(−)-isomer of (IX; R = H), the *p*-phenylphenacyl ester of which was enantiomeric with the ester obtained as described above from the degradation sequence. Identity was confirmed by the usual physical criteria and by mixing the enantiomers to furnish the racemate.



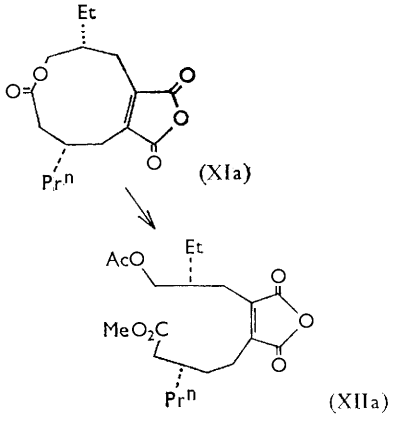
The absolute configuration of the half-amide (IX; R = H) was determined by degradation by the Hofmann rearrangement [10] followed by oxidation to ethylsuccinic acid of known absolute configuration.[11] In this way the (*R*)(−)-half-amide [enantiomer of (IX; R = H), the half-amide from the ketone (IV; X = H_2)] gave (*S*)(−)-ethylsuccinic acid. Identity was again confirmed by mixing with an authentic specimen of (*R*)(+)-ethylsuccinic acid [11] to form the racemate. Ethylsuccinic acid itself was resolved with strychnine.

The isolation of the n-propylsuccinic acid and of the half-amide of β-ethylglutaric acid is consistent with two structures for the original ketone (IV; X = H_2) and (X). The

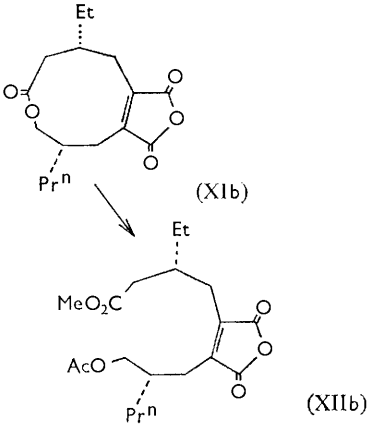
[9] C. R. Stephens, L. H. Conover, R. Pasternak, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1954, **76**, 3568.
[10] E. S. Wallis and J. F. Lane, *Org. Reactions*, **3**, 267.
[11] M. Matell, *Arkiv. Kemi*, 1952, **5**, 17.

absolute configuration of the n-propyl group must be as shown in (IV; X = H_2) or in (X). The *X*-ray crystallography work [3] proves that the ethyl and n-propyl groups must be *cis* with respect to the nine-membered ring. It follows then that enantiomeric half-amides (IX; R = H) would be obtained from (IV; X = H_2) and from (X). The enantiomer expected from (IV; X = H_2) is that obtained. There is an objection to this argument in that epimerisation of the n-propyl group could have occurred in the degradation sequence. To rule out this possibility we examined carefully the ozonolysis of byssochlamic acid. Ozonolysis of the tetrasodium salt in aqueous solution at room temperature for 20 hr. gave (*S*)(−)-ethylsuccinic acid (0·7%), β-ethylglutaric acid (0·7%), and (*S*)(−)-n-propylsuccinic acid (VII) (0·7%). The configuration of the last acid confirms the interpretation of our degradational sequence given above.

The second ketone (V) from byssochlamic acid could not be nitrosated, but with peroxytrifluoroacetic acid [12] it gave a crystalline lactone (XI). Prolonged alkaline hydrolysis, methylation, and acetylation afforded the ester (XII), which showed no vinyl protons in its n.m.r. spectrum. Ozonolysis of this ester gave a mixture of acidic esters. A portion was methylated and analysed by gas chromatography. The volatile part contained the esters of β-ethylglutaric (42%), β-n-propylglutaric (32%), ethylsuccinic (6%), and n-propylsuccinic (7%) acids. On hydrolysis and chromatography over silica, β-ethyl- and β-n-propyl-glutaric acids could be identified, the latter acid as its *p*-phenylphenacyl ester. The only possible explanation of these results is that the lactone (XI) is, in fact, a complex of isomers (XIa) and (XIb) giving as derivatives the esters (XIIa) and (XIIb).



(XIIa)



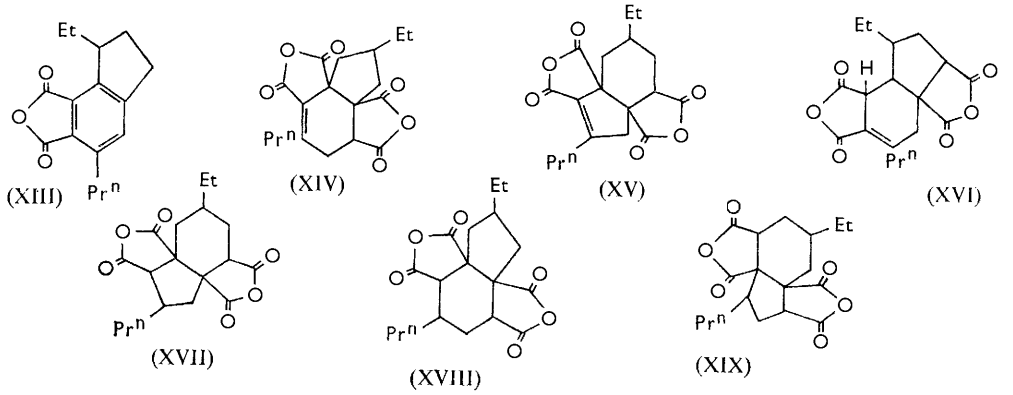
(XIIb)

The early work on byssochlamic acid included experiments on dehydrogenation of the compound over platinum.[4] A major product, $C_{16}H_{18}O_3$, was studied further,[13] and it was shown that oxidation with nitric acid gave benzenepentacarboxylic acid. We have repeated the preparation of the compound $C_{16}H_{18}O_3$. It is a substituted phthalic anhydride showing a single aromatic proton at τ 2·72, not significantly different in position from that found in benzene. We, therefore, assigned this proton as *meta* or *para* to the anhydride grouping. A proton *ortho* to an electronegative grouping should be shifted downfield.[14] Since the dehydrogenation product, $C_{16}H_{18}O_3$, did not react with perphthalic acid, the double bond equivalent not accounted for by the benzene ring and the phthalic anhydride grouping was assumed to be due to a carbon ring. Kuhn–Roth oxidation gave two mol. of volatile acid. Since the n.m.r. spectrum showed that neither methyl nor ethyl groups were attached to the benzene ring in the dehydrogenation product, and since tertiary or secondary methyl groups were, on the same grounds, not present, we were led to formulate

[12] W. F. Sager and A. Duckworth, *J. Amer. Chem. Soc.*, 1955, **77**, 188.
[13] J. W. Cook, J. D. Loudon, and R. P. Paton, unpublished observations; see R. P. Paton, Ph.D. Thesis, Glasgow, 1954.
[14] L. M. Jackman, "Nuclear Magnetic Resonance Spectroscopy," Pergamon, London, 1959.

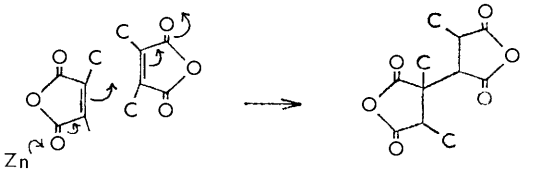
the compound as in (XIII). The position of attachment of the ethyl group in the hydrindane ring was, however, certain only when the full constitution of byssochlamic acid became known.

Sublimation of byssochlamic acid over alumina gave an isobyssochlamic acid.[13] This compound is most readily prepared by heating byssochlamic acid in a sealed tube at 210°. From spectroscopic measurements it was clear that isobyssochlamic acid was a bicyclic bis-anhydride containing an itaconic anhydride type of chromophore. From n.m.r. evidence the ethylenic linkage must be fully substituted. Dehydrogenation of isobyssochlamic acid over platinum gave the phthalic anhydride (XIII). We have already discussed various possible formulæ for isobyssochlamic acid.[2] Since the constitution of this substance has not been unambiguously established, and, indeed, is not important now that the structure of byssochlamic acid itself has been finally settled, we shall here abbreviate all discussion of the problem and merely state [2] that three formulæ [(XIV), (XV), and (XVI)] appear to be reasonable. The matter should be finally clarified by *X*-ray investigations at present in progress.[3] Of course, (XIV) and (XV) would require a further rearrangement during the dehydrogenation to (XIII), but this is not unreasonable.



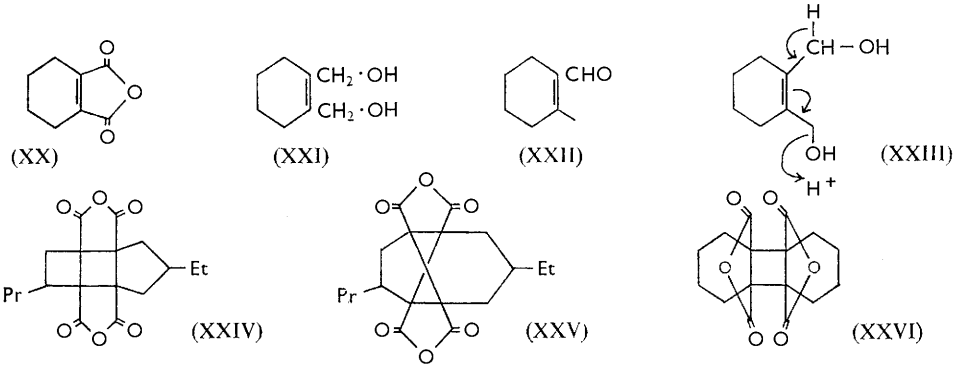
The attachment of the n-propyl group to the ethylenic linkage in isobyssochlamic acid was demonstrated as follows. Allylic substitution with *N*-bromosuccinimide gave a monobromo-derivative, whose n.m.r. spectrum showed absorption due to CHBr at 4·14 τ as a symmetrical triplet ($J = 4$ c./sec.). The hydrogen of CHBr must therefore be split by a freely rotating methylene group. This is possible only if the system CH_3–CH_2–CHBr– is present. Each peak of the triplet was further split into a doublet ($J = 1$ c./sec.) by long-range coupling. The constitution (XVI) would provide an allylic proton [see (XVI)] in the correct relationship to explain the additional splitting. Allylic bromination of isobyssochlamic acid with two mol. of *N*-bromosuccinimide gave a dibromo-derivative.

The earlier workers [4] showed that reduction of byssochlamic acid with zinc dust in acetic acid containing some aqueous hydrochloric acid gave a dihydro-derivative, isolated as its hydrate. Repetition of this work afforded a saturated anhydride-dicarboxylic acid of the expected composition. When heated with acetyl chloride this furnished a dihydrobyssochlamic acid from which the original dicarboxylic acid could be recovered by dissolution in alkali and acidification. As we have discussed,[2] this reductive cyclisation of byssochlamic acid can best be explained by the mechanistic scheme:



Ignoring constitutions containing four membered rings, there are three possible formulæ [(XVII), (XVIII), and (XIX)] for dihydrobyssochlamic acid. In an attempt to distinguish between these formulæ, dihydrobyssochlamic acid hydrate was heated with platinum at 300°, with the intention of effecting dehydrogenation. Instead an isomer of dihydrobyssochlamic acid was obtained. This compound, which was also formed when the platinum was omitted, is probably a stereoisomer. Neither of these dihydrobyssochlamic acids was identical with the compound obtained by hydrogenation of isobyssochlamic acid.

In our earlier attempts to effect the oxidative degradation of byssochlamic acid we planned to investigate the ozonolysis of its lithium aluminium hydride reduction product. From the infrared spectrum of the reduction product it was obvious that it was a complex mixture. In order to understand the possible reduction products better we turned to a study of the reduction of a simpler model compound (XX). Separation of the reaction product into acidic and neutral fractions gave, in the latter fraction, the diol (XXI), characterised as its bis-3,5-dinitrobenzoate. Although the neutral fraction showed no carbonyl absorption in the infrared it gave, when warmed with the 2,4-dinitrophenylhydrazine reagent, the 2,4-dinitrophenylhydrazone of (XXII).[15] Clearly, the acidity of the reagent is sufficient to transform the diol (XXI) into (XXII) by the obvious acid-catalysed process (XXIII, see arrows). The acidic fraction was not studied further. It was, however, clear that the approach to the degradation of byssochlamic acid involving reduction prior to ozonolysis was not attractive.



We also studied briefly the photochemistry of byssochlamic acid. Irradiation with ultraviolet light in tetrahydrofuran gave a saturated photobyssochlamic acid. By analogy with the known photodimerisation of maleic anhydride [16] we formulate this compound as (XXIV) or (XXV). The stability to pyrolysis appears to favour the latter structure. In a model experiment under the same conditions the tetrahydrophthalic anhydride (XX) afforded the dimer (XXVI). This compound, like photobyssochlamic acid, titrated as a dicarboxylic acid. Acidification of the alkaline solution gave a tetracarboxylic acid, characterised as the crystalline tetramethyl ester. There is analogy for polycarboxylic acids which do not titrate for the correct number of carboxyl groups.[17]

Experimental

Melting points were taken on a Kofler block. Unless otherwise specified, optical rotations were measured in chloroform, ultraviolet spectra in ethanol, and infrared spectra in chloroform. N.m.r. spectra were taken at room temperature on *ca.* 10% w/v solutions in deuterochloroform on a Varian A 60 spectrometer on permanent loan from the Wellcome Trust. Gas

[15] M. Mousseron and M. Mousseron-Canot, *Bull. Soc. chim. France*, 1956, 1220.
[16] G. W. Griffin, A. F. Velturo, and K. Furukawa, *J. Amer. Chem. Soc.*, 1961, **83**, 2725.
[17] R. C. Cookson and M. E. Trevett, *J.*, 1956, 3864.

chromatograms were run on a Pye Argon chromatograph using a polyethylene glycol adipate column at 150° and with a gas flow rate of 20 ml./min. The working up of reactions by extractive procedures involved washing the organic phase with saturated sodium chloride solution, drying with sodium sulphate, and evaporation under reduced pressure. Acidification of reaction mixtures was, unless otherwise stated, with hydrochloric acid (2·0N) to Congo Red.

*Byssochlamic Acid Bis-*p*-bromophenylhydrazide* (II).—*p*-Bromophenylhydrazine hydrochloride (160 mg.) in water (10 ml.) was basified with aqueous sodium hydroxide (0·1N; 6·35 ml.), extracted with ether, and the solvent removed *in vacuo*. The crystalline *p*-bromophenylhydrazine in chloroform (3·0 ml.) containing byssochlamic acid (60 mg.) was left at 0° for 2 days. Crystallisation of the ppt. (82 mg.) from ethanol–light petroleum (b. p. 60—80°) gave the *bis*-p-*bromophenylhydrazide* (II) as large plates, m. p. 164—166°, $[\alpha]_D$ −97° (*c* 0·30), $\lambda_{max.}$ 236, 292 mμ (ε 41,000, 3100), $\nu_{max.}$ (Nujol) 1780, 1720 cm.$^{-1}$ (Found: C, 54·1; H, 5·05; Br, 23·3; N, 7·8. $C_{30}H_{30}Br_2N_4O_4$ requires C, 53·7; H, 4·5; Br, 23·8; N, 8·4%).

Degradation of Byssochlamic Acid to the Ketones (IV; X = H_2) *and* (V).—Byssochlamic acid (1·0 g.) in warm aqueous sodium hydroxide (0·2N; 100 ml.) was cooled to room temperature, titrated with hydrochloric acid (4·0N) to pH 10, and treated with hydroxylamine hydrochloride (500 mg.) for 14 hr. at room temperature. Acidification with hydrochloric acid (4·0N), extraction into chloroform, and then re-extraction of the *N*-hydroxy-imide (III; R = H) as the cherry red anion with saturated aqueous sodium hydrogen carbonate gave a solution of (III; R = H) free from unchanged byssochlamic acid. Acidification with hydrochloric acid (4·0N), extraction into chloroform, drying (Na_2SO_4), and removal of the solvent *in vacuo* at 50° gave byssochlamic acid mono-*N*-hydroxy-imide (III; R = H) as a foam (1·10 g.), $\lambda_{max.}$ 214, 232 mμ (ε 9800, 12,500), $\nu_{max.}$ (Nujol) 1840 and 1770 (anhydride), and 1800, 1720, 1660, and 3200 (*N*-hydroxy-imide) cm.$^{-1}$. The *N*-hydroxy-imide in dry pyridine (5 ml.) was treated with toluene-*p*-sulphonyl chloride (1·8 g.) at 0° overnight. The solution was poured into ice–water (250 ml.). After 2 hr., acidification with hydrochloric acid (1·0N) and extraction into chloroform (200 ml.) gave, after evaporation *in vacuo*, the mono-*N*-toluene-*p*-sulphonyloxy-derivative as a foam (1·30 g.). This compound, which was homogeneous on chromatography, in ethanol (12 ml.) and aqueous potassium hydroxide (1·0N; 50 ml.) was heated on a steam-bath under nitrogen for 3 hr. (no further evolution of ammonia). The solution was extracted with chloroform (100 ml.) and the extract discarded. It was then acidified and again extracted with chloroform (200 ml.). Removal of the solvent *in vacuo* gave a mixture of ketones (550 mg.). Chromatography over silica gel (B.D.H. acid-washed; 35 g.), eluting with benzene, gave the *ketone* (IV; X = H_2), m. p. 110° (220 mg.) (from aqueous ethanol), $[\alpha]_D$ −79° (*c* 0·90), $\lambda_{max.}$ 215, 255 mμ (ε 5000, 4600 in cyclohexane), $\nu_{max.}$ 1859, 1761, 1667, 1695 cm.$^{-1}$ (Found: C, 69·0; H, 8·05. $C_{16}H_{22}O_4$ requires C, 69·05; H, 8·0%). The ketone gave a positive Zimmermann test.

Further elution with benzene containing ether (15% v/v) gave the *ketone* (V), m. p. 161° (90 mg.) (from aqueous ethanol), $[\alpha]_D$ +6° (*c* 0·95), $\lambda_{max.}$ 215, 255 mμ (ε 4600 and 5500 in cyclohexane), $\nu_{max.}$ 1845, 1761, 1685, 1658 cm.$^{-1}$ (Found: C, 68·75; H, 7·55%). This ketone did not give a positive Zimmermann test.

Both ketones were recovered unchanged under the alkaline conditions required for their genesis.

Preparation of the Hydroxyimino-ketone (IV; X = NOH).—The ketone (IV; X = H_2) (243 mg.) in dry ether (30 ml.) was treated with hydrogen chloride gas at 0° with stirring for 5 min. Pentyl nitrite (redistilled; 0·5 ml.) in dry ether (10 ml.) was added dropwise during 1 hr., the gas stream being continued as before and then for a further 1 hr. Removal of the solvent *in vacuo*, and crystallisation from chloroform–light petroleum (b. p. 40—60°), gave the *hydroxyimino-ketone* (IV; X = NOH) as needles (210 mg.), m. p. 171—172°, $[\alpha]_D$ +445° (*c* 1·00), $\lambda_{max.}$ 207, 239, 267 mμ (ε 9000, 8500, 4200) shifted to $\lambda_{max.}$ 230 mμ (ε 8500) by aqueous potassium hydroxide (1 drop; 0·1N), $\nu_{max.}$ (Nujol) 1845, 1775, 1680, 1630, 3300 cm.$^{-1}$ (Found: C, 63·05; H, 6·85. $C_{16}H_{21}NO_5$ requires C, 62·5; H, 6·9%).

Preparation of the Nitrile-Acid (VI) *and its Further Transformations.*—The hydroxyimino-ketone (IV; X = NOH) (50 mg.) in aqueous potassium hydroxide (0·5N; 4·0 ml.) was shaken at room temperature with finely powdered toluene-*p*-sulphonyl chloride (104 mg.) for 15 hr. Filtration, saturation with sodium chloride, acidification, and extraction into ether (150 ml.) gave the nitrile-acid (VI) as an oil (30 mg.), $\lambda_{max.}$ 210, 251 mμ (ε 9000, 3900), $\nu_{max.}$ 2600—3300, 2300, 1823, 1767, 1708 cm.$^{-1}$. With ethereal diazomethane this gave the *methyl ester*, b. p.

95°/10^{-4} mm., $[\alpha]_D$ −7° (*c* 1·80), $\lambda_{max.}$ 210, 255 mμ (ε 9000, 4000), $\nu_{max.}$ 1855, 1820, 1770, 1730, 1280, 2300 $cm.^{-1}$ (Found: C, 63·7; H, 7·35. $C_{17}H_{23}NO_5$ requires C, 63·55; H, 7·2%).

The methyl ester (97 mg.) in ethyl acetate (AnalaR; 20 ml.) was ozonised at 0° for 7 hr. (90% disappearance of the peak at 255 mμ). The ozonide was worked up by either of two different procedures, A and B.

Procedure A. The ozonide was treated at 0° with potassium hydroxide (1·0N; 3·0 ml.) and hydrogen peroxide (30%; 2·0 ml.) and left at ambient temperature for 20 min. A trace of platinum oxide was added to destroy excess peroxide and the solution left for 10 hr. Warming on a steam-bath for 30 min., saturation with sodium chloride, acidification, and continuous extraction with ether for 24 hr. gave a mixture of acids (54 mg.). A small portion (3 mg.) was converted into methyl esters (diazomethane) and analysed by gas chromatography. Two components in approximate 2 : 1 ratio could be detected as well as dimethyl oxalate (<5%). The mixed acids were separated as follows. A partition column of silica gel (B.D.H. chromatography grade; 45 g.) was prepared [18] in chloroform, and the mixed acids were introduced in the minimum of the same solvent. Elution with chloroform containing 2% of butanol (saturated with water) gave the minor acid. Analogous elution using 3% of butanol furnished the major acid. The latter (30 mg.) was sublimed in a high vacuum (95°/10^{-4} mm.), to give (−)-n-propylsuccinic acid (20 mg.), m. p. 103—104° [from benzene–light petroleum (b. p. 60—80°)], $[\alpha]_D$ −18° (*c* 0·6), infrared spectrum (Nujol) identical with that of authentic (+)-n-propylsuccinic acid, m. p. 104—105°, $[\alpha]_D$ +18° (*c* 1·0).[19] The corresponding dimethyl ester (diazomethane) ran in a gas chromatogram at the same position as (±)-dimethyl n-propylsuccinate. Finally, mixing equal amounts (3·83 mg.) of the two enantiomers gave, after recrystallisation from benzene–light petroleum (b. p. 40—60°), (±)-n-propylsuccinic acid, m. p. 92°, identified by mixed m. p. and infrared spectrum in Nujol. The minor component (20 mg.) was heated with conc. hydrochloric acid (2·0 ml.) at 95° for 3 hr. Removal of the aqueous acid *in vacuo*, extraction into chloroform, and sublimation of the oily extract (7 mg.) at 95°/10^{-4} mm. gave a crystalline product [from benzene–light petroleum (b. p. 60—80°)], β-ethylglutaric acid, identified by m. p. and mixed m. p. Identity was confirmed by methylation (diazomethane) and comparative gas chromatography.

Procedure B. The ozonide was treated at 0° with aqueous potassium hydroxide (1·0N; 3·0 ml.) and hydrogen peroxide (30%; 2·0 ml.) and then heated on a steam-bath for 8 hr. The solution was cooled, acidified, and continuously extracted with ether for 24 hr., to furnish the mixed acids (60 mg.) as a gum. This gum, in absolute ethanol (5·0 ml.), was titrated with aqueous potassium hydroxide (0·1N). The warmed solution, adjusted to pH 5—6, and *p*-phenylphenacyl bromide (198 mg.) in ethanol (2·0 ml.) were heated on a steam-bath for 3 hr. Slow cooling gave the *bis-p-phenylphenacyl ester* of (−)-n-propylsuccinic acid. Purified by chromatography over alumina (Grade III, neutral; 6·0 g.) in benzene, this (20 mg.) had m. p. 144—147°, $[\alpha]_D$ +34° (*c* 1·2) (Found: C, 77·1; H, 5·9. $C_{35}H_{32}O_6$ requires C, 76·6; H, 5·9%). The infrared spectrum was identical with that of the corresponding bis-ester of authentic (+)-n-propylsuccinic acid (see above). The filtrate, after removal of the bis-*p*-phenylphenacyl ester, was diluted with water and extracted with chloroform, to give a gum (90 mg.) which was chromatographed over alumina (Grade III, neutral; 6·0 g.) in benzene. Elution with benzene–chloroform (2 : 1) gave the p-*phenylphenacyl ester* of (+)-γ-carbamoyl-β-ethylbutyric acid (15 mg.), m. p. 137—139° [from benzene–light petroleum (b. p. 40—60°)], $[\alpha]_D$ +15° (*c* 0·60), $\gamma_{max.}$ (Nujol) 1740, 1695, 1660, 1633, 1600 $cm.^{-1}$ (Found: C, 71·55; H, 6·55. $C_{21}H_{23}NO_4$ requires C, 71·35; H, 6·55%). The infrared spectrum of this derivative was identical with that of the corresponding ester of (−)-γ-carbamoyl-β-ethylbutyric acid. The identity was confirmed by mixing equal amounts of the (+)- and (−)-forms to give, after recrystallisation from benzene, the *p*-phenylphenacyl ester of (±)-γ-carbamoyl-β-ethylbutyric acid (m. p., mixed m. p., and infrared spectrum).

Preparation and Reactions of the Keto-lactone (XI).—The ketone (V) (200 mg.) in trifluoroacetic anhydride (4·55 g., 3·0 ml.) at −12° was stirred during addition of hydrogen peroxide (80%; 1·12 g., 0·9 ml.) during 30 min. and then for a further 30 min. at 0° before being left for 48 hr. at 0°. The solution was poured into ice–water and extracted with chloroform. Removal of the solvent and crystallisation from aqueous ethanol gave the *keto-lactone* (XI) (83 mg.), m. p. 100—103°, $[\alpha]_D$ +5° (*c* 1·00), $\lambda_{max.}$ 209, 256 mμ (ε 4300, 4500 in cyclohexane),

[18] C. S. Marvel and R. D. Rands, *J. Amer. Chem. Soc.*, 1950, **72**, 2642.
[19] P. Clutterbuck, H. Raistrick, and F. Reuter, *Biochem. J.*, 1937, **31**, 987.

$\nu_{max.}$ 1835, 1773, 1706, 1160 cm.$^{-1}$ (Found: C, 65·2; H, 7·25. $C_{16}H_{22}O_5$ requires C, 65·3; H, 7·55%).

The keto-lactone (XI) (83 mg.) in aqueous sodium hydroxide (4·0N, 2 ml.) under nitrogen was heated on a steam-bath for 5 hr., acidified, and extracted with chloroform. Removal of the solvent *in vacuo* gave the hydroxy-acid (70 mg.) as an oil, $\lambda_{max.}$ 210, 253 mμ (ε 6000, 4500), $\nu_{max.}$ 3300, 1820, 1760, 1705 cm.$^{-1}$. Treatment with ethereal diazomethane afforded the methyl ester, which with acetic anhydride (0·6 ml.), chloroform (1·0 ml.), and pyridine (2 drops) at 0° gave, on working up in the usual way, the *acetate-ester* (XII) (75 mg.), b. p. 110°/10^{-4} mm., $[\alpha]_D$ 0° (*c* 2·0), $\lambda_{max.}$ 253 mμ (ε 4500), $\nu_{max.}$ 1820, 1760, 1725, 1620 cm.$^{-1}$ (Found: C, 62·45; H, 8·25. $C_{19}H_{28}O_7$ requires C, 61·95; H, 7·65%).

The acetate-ester (XII) (75 mg.) in ethyl acetate (AnalaR; 30 ml.) was ozonised at 0° for 4 hr. (disappearance of the maximum at 253 mμ). The solvent was removed *in vacuo* at room temperature and the residue treated with (premixed) aqueous sodium hydroxide (1·0N; 3·0 ml.) and hydrogen peroxide (30%; 3·0 ml.) and heated on a steam-bath for 2 hr. Acidification, saturation with sodium chloride, and continuous extraction with ether for 12 hr. gave an acidic oil (53 mg.). Methylation of a portion (5 mg.) with ethereal diazomethane and gas chromatography showed the presence of the following components: dimethyl ethylsuccinate (6%), dimethyl n-propylsuccinate (7%), dimethyl β-ethylglutarate (42%), dimethyl β-n-propylglutarate (32%), and an unknown component (13%). Each compound was identified by the appropriate mixed chromatogram.

The β-substituted glutaric acids were further characterised as follows. The total combined ozonolysis products from three separate ozonolyses and further processing as above (132 mg.) were chromatographed over silica gel (175 g.) in chloroform.[18] Elution with chloroform containing 7% of butanol (water-saturated), sublimation at 100°/10^{-4} mm., and crystallisation from carbon tetrachloride furnished β-ethylglutaric acid (3 mg.), identified by m. p., mixed m. p., and infrared spectrum. Elution with chloroform containing 6% of butanol gave β-n-propylglutaric acid (12 mg.). This was purified by sublimation at 95—100°/10^{-4} mm. but did not crystallise. It was therefore converted into its bis-*p*-phenylphenacyl ester as in the preceding section. Chromatography of the product over alumina (Grade III, neutral; 6·0 g.) in benzene and crystallisation from benzene–light petroleum (b. p. 60—80°) furnished (2 mg.) the bis-ester of β-n-propylglutaric acid (m. p., mixed m. p., and infrared spectrum).

Ozonolysis of Byssochlamic Acid as the Tetrasodium Salt.—Byssochlamic acid (444 mg.) in aqueous sodium hydroxide (1·0N; 7·0 ml.) was titrated to pH 10 with dilute hydrochloric acid, and the volume adjusted to 75 ml. Ozonolysis at 0° for 20 hr. (disappearance of band at 232 mμ), addition of aqueous sodium hydroxide (1·0N; 6 ml.) and hydrogen peroxide (30%; 6 ml.), heating on a steam-bath for 2 hr., acidification, saturation with sodium chloride, and continuous extraction with ether gave a yellow oil. Digestion of this with chloroform afforded oxalic acid (60 mg.) and soluble acids. The ozonolysis was repeated until 2·5 g. of byssochlamic acid had been degraded. The chloroform-soluble acids were chromatographed over silica gel (B.D.H.; 300 g.) as before.[18] Oxalic acid appeared in all fractions containing up to 8% of butanol. Fractions (200 mg.) eluted with 6—8% of butanol also contained, by gas chromatography of their methyl esters, n-propyl- and ethyl-succinic acids and also β-ethylglutaric acid. Further chromatography over silica gel gave a mixture of n-propylsuccinic and β-ethylglutaric acids (100 mg.), as well as ethylsuccinic acid (10 mg.), $[\alpha]_D$ −3° (*c* 1·0). After model studies with mixtures of n-propylsuccinic and β-ethylglutaric acids, it was found that these two acids could be separated by chromatography in carbon tetrachloride–butanol over silica gel. Thus, the mixed acids (100 mg.) were chromatographed [18] over silica gel (B.D.H.; 100 g.), eluting with carbon tetrachloride–butanol. Elution with 7% of butanol (water-saturated), followed by sublimation in a high vacuum and crystallisation from benzene–light petroleum (b. p. 60—80°), gave (−)-n-propylsuccinic acid (10 mg.), m. p. and mixed m. p. 104—105°, $[\alpha]_D$ −18° (*c* 1·0). Further elution with the same solvent mixture gave, after sublimation in a high vacuum and crystallisation from carbon tetrachloride, β-ethylglutaric acid (5 mg.) (m. p. and mixed m. p.).

γ-Carbamoyl-β-ethylbutyric Acid and Derivatives.—β-Ethylglutaric acid [20] anhydride (1·0 g.) in benzene (10 ml.) was added dropwise to well stirred aqueous ammonia (14 ml.; 2·0N) at 0°. Acidification with dilute hydrochloric acid, filtration (1·0 g.), and crystallisation from water gave *γ-carbamoyl-β-ethylbutyric acid* as fine needles, m. p. 132—133°. Sublimation at 120°/10^{-4} mm. gave an analytical specimen, m. p. 133—134°, $\nu_{max.}$ (Nujol) 1708, 1670, 1590, 2600—

[20] F. B. Thole and J. F. Thorpe, *J.*, 1911, **99**, 429.

3500 cm.$^{-1}$ (Found: C, 53·1; H, 8·1; N, 9·0. $C_7H_{13}NO_3$ requires C, 52·8; H, 8·25; N, 8·8%). The derived p-*phenylphenacyl ester*, prepared in the usual way, crystallised from benzene as fine needles, m. p. 134—135°, $\nu_{max.}$ 3400, 3200, 1725, 1685, 1640, 1600 cm.$^{-1}$ (Found: C, 71·8; H, 6·9. $C_{21}H_{23}NO_4$ requires C, 71·4; H, 6·5%).

The acid (30 g.) treated in methanolic solution with excess of ethereal diazomethane gave *methyl γ-carbamoyl-β-ethylbutyrate*, plates [from ether–light petroleum (b. p. 40—60°)], m. p. 55—58°, $\nu_{max.}$ 1725, 1680, 1600, 3450, 3600 cm.$^{-1}$ (Found: C, 55·25; H, 8·8; N, 8·0. $C_8H_{15}NO_3$ requires C, 55·45; H, 8·75; N, 8·1%).

This ester (31 g.) in dry pyridine (200 ml.) was treated at 0° with toluene-*p*-sulphonyl chloride (freshly recrystallised; 175 g.) for 90 min. (solidification). The mixture was left at ambient temperature for 2·5 hr., poured into ice–water (1 l.), and left for 2 hr. The solution was acidified at 0° with conc. hydrochloric acid added dropwise with stirring, and extracted with ether, to furnish a yellow oil (25 g.), $\nu_{max.}$ 1735, 2260 cm.$^{-1}$. Distillation of a portion (500 mg.) twice gave *methyl γ-cyano-β-ethylbutyrate*. b. p. 60°/10^{-4} mm., $\nu_{max.}$ (film) 1735, 2260 cm.$^{-1}$ (Found: C, 61·95; H, 8·45; N, 8·75. $C_8H_{13}NO_2$ requires C, 61·9; H, 8·45; N, 9·05%).

After preliminary experiments this methyl ester was hydrolysed as follows. To the ester (31 g.) in ethanol (400 ml.) and water (400 ml.) aqueous potassium hydroxide (1·0N; 400 ml.) was added with stirring at 0° during 30 min., and the stirring continued at 0° for a further 30 min. Cautious acidification at 0° with conc. hydrochloric acid, extraction into ether (1·5 l.), and distillation of the product *in vacuo* gave *γ-cyano-β-ethylbutyric acid* (21 g.), b. p. 95—105°/10^{-4} mm., n_D^{24} 1·4511, $\nu_{max.}$ (film) 1705, 2280, 2700—3300 cm.$^{-1}$. Since this acid did not crystallise, its constitution was confirmed as follows. The cyano-acid (112 mg.) in aqueous sodium hydroxide (1·0N; 2·0 ml.) and hydrogen peroxide (30%; 1·0 ml.) was kept at 60° for 4 hr., cooled to room temperature, acidified with dilute hydrochloric acid (1·0N), and the solvent (water) removed *in vacuo*. Crystallisation of the residue from water gave γ-carbamoyl-β-ethylbutyric acid (see above) (54 mg.).

γ-Cyano-β-ethylbutyric acid was resolved in the following way.[21] Quinine methochloride (14 g.) and silver oxide (15 g.) in water (70 ml.) were shaken for 3 hr. The mixture was filtered through Celite and the clear solution titrated against γ-cyano-β-ethylbutyric acid (6·0 g.) in ethanol (60 ml.) to pH 9—10. The solvent was removed *in vacuo*, and the residue dried azeotropically with benzene, taken up in acetonitrile (70 ml.), filtered through Celite, and left at room temperature. The crystalline precipitate (6·0 g.), m. p. 162—166°, $[\alpha]_D$ −157° (*c* 1·2 in water), was recrystallised from acetonitrile three times (4·0 g.), m. p. 166—168°, $[\alpha]_D$ −154° (*c* 1·4 in water). This salt in water (8 ml.) was acidified with dilute sulphuric acid (1·0N) and extracted with ether, to furnish (+)-γ-cyano-β-ethylbutyric acid (1·1 g.), b. p. 95—100°/10^{-4} mm., $[\alpha]_D$ +28° (*c* 5·2), $\nu_{max.}$ (film) 1705, 2280 cm.$^{-1}$.

The redistilled acid (491 mg.) suspended in water (2·5 ml.) was treated at room temperature with aqueous sodium hydroxide (1·0N; 10 ml.) and hydrogen peroxide (30%; 5·0 ml.) and then kept at 60° for 3 hr. Working up as for the racemic example (see above) gave (−)-*γ-carbamoyl-β-ethylbutyric acid* (250 mg.), m. p. 113—114° (from water), $[\alpha]_D$ −2° (*c* 1·1 in water), −2° (*c* 0·8 in ethanol) (Found: C, 53·0; H, 8·4; N, 8·9. $C_7H_{13}NO_3$ requires C, 52·8; H, 8·25; N, 8·8%). This acid was further characterised as its p-*phenylphenacyl ester*. Prepared in the usual way and purified by chromatography over alumina (Grade III, neutral; 5 g.) in benzene and crystallised from the same solvent, this had m. p. 137—139°, $[\alpha]_D$ −15° (*c* 0·8), $\nu_{max.}$ (Nujol) 1740, 1695, 1660, 1600, 3400, 3200 cm.$^{-1}$ (Found: C, 72·0; H, 6·35; N, 3·85. $C_{21}H_{23}NO_4$ requires C, 71·35; H, 6·55; N, 3·95%).

(−)-γ-Carbamoyl-β-ethylbutyric acid (144 mg.) in aqueous potassium hydroxide (1·0N; 4·0 ml.) was treated with bromine (166 mg.) at room temperature for 30 min. and then on a steam-bath for 2 hr. The resulting solution was oxidised at room temperature with aqueous potassium permanganate (1·0N; 3·0 ml.) added with stirring during 1·5 hr. The excess of oxidant was destroyed with sulphur dioxide, the solution saturated with sodium chloride and then extracted with ether. Chromatography of the acidic product over silica gel [18] (B.D.H.; 55 g.), eluting with chloroform–butanol mixtures, gave (−)-ethylsuccinic acid (27 mg.), m. p. [from benzene–light petroleum (b. p. 60—80°)] 94—96°, $[\alpha]_D$ −10° (*c* 1·1), identified by mixed m. p., infrared spectrum, and by gas chromatography of the derived dimethyl ester. The identity was confirmed by mixing with (+)-ethylsuccinic acid to obtain (±)-ethylsuccinic acid.

Dehydrogenation of Byssochlamic Acid.—Byssochlamic acid (423 mg.) with platinum black

[21] R. T. Major and J. Finkelstein, *J. Amer. Chem. Soc.*, 1941, **63**, 1368.

(84 mg.) was heated under nitrogen at 300—310° for 2 hr. (no further gas evolution). Ether extraction of the melt gave a resin which was extracted with light petroleum (b. p. 60—80°). The extracted material was chromatographed over silica gel (B.D.H. acid washed; 15 g.). Elution with benzene–light petroleum (b. p. 40—60°) (70 : 40 v/v) gave the *dehydrogenation product* (XIII), m. p. 90—91° [from light petroleum (b. p. 40—60°)] (20 mg.), $[\alpha]_D$ ±0° (*c* 1·1), $\lambda_{max.}$ 224, 233 mμ (ε 26,000, 18,000), $\nu_{max.}$ 1835, 1767 cm.$^{-1}$ [Found: C, 74·65; H, 7·15%; *M* (Rast), 247. $C_{16}H_{18}O_3$ requires C, 74·4; H, 7·05%; *M*, 258]. Kuhn–Roth oxidation gave 1·9 mol. of volatile acid. The compound gave no colour with tetranitromethane and consumed no perphthalic acid.

Isobyssochlamic Acid and its Derivatives.—Byssochlamic acid (513 mg.) was kept at 210° *in vacuo* for 24 hr. Crystallisation of the product from chloroform–ether gave isobyssochlamic acid,[13] fine needles (289 mg.), m. p. 153°, $[\alpha]_D$ −27° (*c* 0·9), $\lambda_{max.}$ 207, 236 mμ (ε 6000, 6300), $\nu_{max.}$ 1830, 1810, 1770, 1650 cm.$^{-1}$ (Found: C, 64·85; H, 6·3. Calc. for $C_{18}H_{20}O_6$ C, 65·0; H, 6·05%). This acid (50 mg.) and platinum black (50 mg.) were heated *in vacuo* at 300° for 7·5 hr. Distillation in a high vacuum and crystallisation of the distillate from light petroleum (b. p. 40—60°) gave the dehydrogenation product (XIII) (4 mg.) (m. p. and mixed m. p.).

Isobyssochlamic acid (100 mg.) in aqueous sodium hydroxide (1·0N; 5·0 ml.) was acidified with conc. hydrochloric acid to pH 2 and continuously ether extracted for 17 hr. Crystallisation of the extract from acetonitrile–benzene afforded isobyssochlamic acid hydrate,[13] m. p. 173—183° (decomp.), $[\alpha]_D$ +24° (*c* 1·3 in acetone), $\lambda_{max.}$ 234 mμ (ε 3300), $\nu_{max.}$ (Nujol) 1840, 1780, 1700, 1690 cm.$^{-1}$. On keeping at 190° for 5 min. followed by crystallisation from chloroform–ether, this dicarboxylic acid gave back isobyssochlamic acid (m. p. and mixed m. p.).

Isobyssochlamic acid (75 mg.) was treated with alkali as above and then acidified at 0° with ice-cold hydrochloric acid (2·0N). Continuous ether extraction gave, on cautious evaporation of the ether, isobyssochlamic acid dihydrate, m. p. 165—168° (decomp.), $[\alpha]_D$ +8° (*c* 1·2 in acetone), $\nu_{max.}$ (Nujol) 1718—1680 cm.$^{-1}$. On heating this tetracarboxylic acid *in vacuo* at 150—160° it quickly reverted to isobyssochlamic acid (m. p., mixed m. p., and infrared spectrum).

Isobyssochlamic acid (126 mg.) in glacial acetic acid (AnalaR; 15·0 ml.) was hydrogenated over palladised charcoal (10%; 117 mg.). Crystallisation of the product from benzene–ether gave dihydroisobyssochlamic acid,[13] m. p. 128—130°, $[\alpha]_D$ −16° (*c* 0·9), ε (220 mμ) 250, $\nu_{max.}$ (Nujol) 1840, 1775 cm.$^{-1}$ (Found: C, 64·7; H, 6·8. Calc. for $C_{18}H_{22}O_6$: C, 64·65; H, 6·65%). The compound gave no colour with tetranitromethane. Treatment with aqueous potassium hydroxide (1·0N) on a steam-bath for 50 min., acidification to pH 2, and extraction with ethyl acetate gave, on crystallisation from this solvent, dihydroisobyssochlamic acid hydrate,[13] m. p. 190—197° (decomp.), $\nu_{max.}$ 1828, 1776, 1701, 1689 cm.$^{-1}$ (Found: C, 61·5; H, 6·7. Calc. for $C_{18}H_{24}O_7$: C, 61·35; H, 6·85%). When heated at 190° for 5 min. this dicarboxylic acid gave back dihydroisobyssochlamic acid (m. p. and mixed m. p.).

Bromination of Isobyssochlamic Acid.—Isobyssochlamic acid (202 mg.) in carbon tetrachloride (60 ml.; AnalaR and freshly distilled from *N*-bromosuccinimide) was refluxed with *N*-bromosuccinimide (124 mg., freshly crystallised from water) for 11 hr. After cooling to 0° and filtration of the precipitated succinimide (61 mg.), the solvent was removed *in vacuo* and the product crystallised from benzene–ether to furnish *monobromoisobyssochlamic acid* (85 mg.), m. p. 177—180°, $[\alpha]_D$ −31° (*c* 1·0), $\lambda_{max.}$ 240 mμ (ε 8600 in cyclohexane), $\nu_{max.}$ (Nujol) 1830, 1775, 1665 cm.$^{-1}$ (Found: C, 52·25; H, 4·65; Br, 20·6. $C_{18}H_{19}BrO_6$ requires C, 52·5; H, 4·6; Br, 19·45%).

Isobyssochlamic acid (494 mg.) in carbon tetrachloride (200 ml., see above) was refluxed with *N*-bromosuccinimide (314 mg.) for 13 hr., further *N*-bromosuccinimide (200 mg.) was added, and the refluxing continued for a further 15 hr. Working up as above and chromatography over silica gel (15·0; acid washed) gave, on elution with benzene and crystallisation from benzene–ether, *dibromoisobyssochlamic acid* (50 mg.) as long needles, m. p. 153—155°, $\lambda_{max.}$ 258 mμ (ε 8600 in cyclohexane), $\nu_{max.}$ (Nujol) 1840, 1775 cm.$^{-1}$ (Found: C, 44·4; H, 3·95. $C_{18}H_{18}Br_2O_6$ requires C, 44·1; H, 3·7%). Further elution with benzene gave the monobromo-derivative (150 mg.) already described above (m. p. and mixed m. p).

*Isobyssochlamic Acid Bis-*p*-bromophenylhydrazide.*—Isobyssochlamic acid (206 mg.) and *p*-bromophenylhydrazine (260 mg.; freshly prepared from the hydrochloride) in benzene (20 ml.) were refluxed under nitrogen for 3 hr. The benzene was removed *in vacuo*, the residue taken up in chloroform, washed with dilute hydrochloric acid (1·0N; 25 ml.), with aqueous sodium hydrogen carbonate, and then with water. The chloroform was removed *in vacuo* and the residue

crystallised from chloroform–light petroleum (b. p. 40—60°), to give *isobyssochlamic acid bis-p-bromophenylhydrazide* (125 mg.) as large hexagonal plates, m. p. 140—145°, $[\alpha]_D$ −8° (*c* 0·9), $\nu_{max.}$ (Nujol) 3300, 1770, 1720, 1660 cm.$^{-1}$ (Found: C, 54·2; H, 4·5; Br, 24·5; N, 8·8. $C_{30}H_{30}Br_2N_4O_4$ requires C, 53·7; H, 4·5; Br, 23·8; N, 8·35%).

Dihydrobyssochlamic Acid and its Derivatives.—(*a*) Byssochlamic acid (400 mg.) in glacial acetic acid (AnalaR; 6·0 ml.) was refluxed with zinc dust (4·0 g.), aqueous hydrochloric acid (1·0N; 8 ml.) being added in 8 portions at 1 hr. intervals. Removal of excess zinc gave a filtrate which, on standing at 0° for 2 days, deposited dihydrobyssochlamic acid hydrate [13] (86 mg.), m. p. 233—240° (from water), $[\alpha]_D$ +14° (*c* 0·9 in acetone), ε (220 mμ) 450, $\nu_{max.}$ (Nujol) 2700, 1820, 1775, 1705 cm.$^{-1}$.

(*b*) Byssochlamic acid (296 mg.) in glacial acetic acid (AnalaR; 35 ml.) was shaken for 24 hr. at room temperature with zinc dust (3·1 g.). Chromatography of the product over silica gel (15 g.; acid washed) gave the same dicarboxylic acid on elution with benzene containing 20% v/v of ether.

Dihydrobyssochlamic acid hydrate (103 mg.) in acetyl chloride (5 ml.; redistilled) was refluxed for 4 hr. The excess of reagent was removed *in vacuo* and residue crystallised from benzene–light petroleum (b. p. 60—80°), to furnish dihydrobyssochlamic acid [13] (87 mg.), m. p. 120°, $[\alpha]_D$ −54° (*c* 1·0), ε (220 mμ) 350, $\nu_{max.}$ (Nujol) 1840, 1775 cm.$^{-1}$ (Found: C, 64·5; H, 6·65. Calc. for $C_{18}H_{22}O_6$: C, 64·65; H, 6·65%). This compound (53 mg.) in aqueous potassium hydroxide (4N; 1·5 ml.) was heated on a steam-bath for 1 hr., and the resultant solution acidified and continuously extracted with ether for 18 hr. Crystallisation of the product (55 mg.) from dioxan gave dihydrobyssochlamic acid hydrate (m. p., mixed m. p., and infrared spectrum).

Dihydrobyssochlamic acid hydrate (50 mg.) was heated *in vacuo* at 300° for 8 hr. Crystallisation from benzene–ether gave an *isomer* (30 mg.), m. p. 168—170°, $[\alpha]_D$ +72° (*c* 1·1), ε (220 mμ) 445, $\nu_{max.}$ (Nujol) 1840, 1775 cm.$^{-1}$ (Found: C, 65·1; H, 6·6. $C_{18}H_{22}O_6$ requires C, 64·65; H. 6·65%).

Reduction of Cyclohexene-1,2-dicarboxylic Anhydride with Lithium Aluminium Hydride.—The anhydride (1·0 g.) in dry tetrahydrofuran (10 ml.; freshly distilled from lithium aluminium hydride) was added dropwise to a suspension of lithium aluminium hydride (2·5 g.) in the same solvent (70 ml.) under reflux, and the solution refluxed for 12 hr. Addition of ethyl acetate, washing with saturated aqueous sodium sulphate, filtration, and removal of the solvent gave an oil (940 mg.), $\nu_{max.}$ 3400, 1725 cm.$^{-1}$. The following experiments were carried out on this oily product.

The oil (500 mg.) in dry pyridine (2 ml.) was treated at 0° with 3,5-dinitrobenzoic acid (864 mg.) and toluene-*p*-sulphonyl chloride (755 mg.) for 12 hr. Working up in the usual way and crystallisation from benzene–light petroleum (b. p. 40—60°) gave *1,2-bis-3,5-dinitrobenzoyloxymethylcyclohexene* (361 mg.), m. p. 158—160°, $\lambda_{max.}$ 209, 224 mμ (ε 53,000, 40,500), $\nu_{max.}$ 1730, 1630, 1600 cm.$^{-1}$ (Found: C, 49·85; H, 3·8; N, 10·45. $C_{22}H_{18}N_4O_{12}$ requires C, 49·8; H, 3·4; N, 10·55%). The n.m.r. spectrum in deuterochloroform showed the following signals (τ values): 0·70 (multiplet, aryl protons), 4·53 (sharp singlet, 4 protons, allylic ester methylene groups), 7·58 (broad singlet, 4 protons, allylic ring methylenes), 8·17 (broad singlet, 4 protons, non-allylic ring methylenes).

The oil (436 mg.) in ethanol (10 ml.) was heated with excess of 2,4-dinitrophenylhydrazine reagent for 2·5 hr., cooled, and filtered. The product, chromatographed over kieselguhr–bentonite in the usual way, crystallised from chloroform–ethanol as red needles (75 mg.) of *2-methylcyclohexene-1-carboxyaldehyde 2,4-dinitrophenylhydrazone*, m. p. 190—191°, $\lambda_{max.}$ 388 mμ (ε 30,400) (Found: C, 54·85; H, 5·15; N, 8·25. $C_{14}H_{16}N_8O_4$ requires C, 55·25; H, 5·3; N, 8·4%). The n.m.r. spectrum in deuterochloroform showed a band at 8·04 τ (sharp singlet) due to vinylic methyl, and a singlet at 1·70 (sharp singlet) due to the aldehyde proton (as the 2,4-dinitrophenylhydrazone).

Preparation of Photobyssochlamic Acid and Related Experiments.—Byssochlamic acid (129 mg.) in tetrahydrofuran (250 ml.; freshly distilled from lithium aluminium hydride) was irradiated with a high-pressure mercury lamp (125 w) under nitrogen for 1 hr. (ultraviolet control). The solvent was removed *in vacuo*, to give *photobyssochlamic acid* (91 mg.), m. p. 210—220° (from chloroform–ether) (50 mg.), $[\alpha]_D$ +6° (*c* 1·0), ε (220 mμ) 725, $\nu_{max.}$ (Nujol) 1842, 1821, 1764 cm.$^{-1}$ [Found: C, 65·1; H, 6·15%; *M* (mass spectrum), 332. $C_{18}H_{20}O_6$ requires C, 65·0; H, 6·05%; *M*, 332]. Photobyssochlamic acid (40 mg.) in aqueous potassium

hydroxide (1·0N; 1·0 ml.) was back-titrated with dilute hydrochloric acid (0·1N; 7·5 ml.) to phenolphthalein (Equiv., 160. Calc. for 2-carboxyls: 166). Acidification with conc. hydrochloric acid gave back photobyssochlamic acid (m. p., mixed m. p., and infrared spectrum).

Photobyssochlamic acid (22 mg.), heated *in vacuo* at 310° for 3 hr. gave, after crystallisation from chloroform, only starting material (15 mg.) (m. p., mixed m. p., and infrared spectrum.)

Cyclohexene-1,2-dicarboxylic acid anhydride (1·2 g.) in cyclohexane (200 ml.; AnalaR) was irradiated as above for 3 hr. under nitrogen (ultraviolet control). Removal of the solvent *in vacuo* and crystallisation from benzene afforded the *photodimer* (XXVI) (376 mg.) as cubes, m. p. 277—283° (capillary), $\nu_{max.}$ 1865, 1835, 1790 cm.$^{-1}$ [Found: C, 63·4; H, 5·55%; *M*(ebullioscopic), 313. $C_{16}H_{16}O_6$ requires C, 63·15; H, 5·3%; *M*, 304]. This photodimer (95 mg.) in aqueous potassium hydroxide (1·0N; 2·0 ml.) was back-titrated with aqueous hydrochloric acid (1·0N) to phenolphthalein (Equiv., 140). Acidification with hydrochloric acid (6·0N) and extraction with ether gave a foam (99 mg.), $\nu_{max.}$ 1695 cm.$^{-1}$. Treatment with diazomethane in ether afforded the *tetramethyl ester*, m. p. 132—135° [from benzene–light petroleum (b. p. 40—60°)], $\nu_{max.}$ 1738, 1728 cm.$^{-1}$ (Found: C, 61·8; H, 7·25. $C_{20}H_{28}O_8$ requires C, 60·6; H, 7·1%). Sublimation of a portion of the acidic foam *in vacuo* re-formed the photodimer (XXVI) (m. p., mixed m. p., and infrared spectrum).

The photodimer (XXVI) (80 mg.) was heated *in vacuo* at 300° for 11 hr. Crystallisation from chloroform–cyclohexane gave back starting material (50 mg.). Removal of solvent from the mother-liquors *in vacuo*, and sublimation at 75°/10^{-4} mm., gave cyclohexene-1,2-dicarboxylic acid anhydride (10 mg.) (m. p., mixed m. p., and infrared spectrum).

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