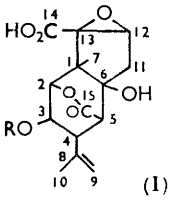
400. *Picrotoxin and Tutin. Part X.*[1]

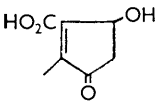
By R. M. Carman, R. G. Coombe, R. B. Johns, and A. D. Ward.

Preparation of the monomethyl ether of apopicrotoxinic dilactone is described, and experiments are reported which uniquely determine the position and nature of the ether system in picrotoxinin.

Part IX [1] described experiments which confirmed the position of the 3-hydroxyl group in α-picrotoxinic acid (I; R = H). Concurrently with those investigations, and with the same objective, an alternative approach was developed *via* the methyl ether of β-bromopicrotoxinic acid.[2] From these experiments a neutral dilactone was isolated and identified as the monomethyl ether of apopicrotoxinic dilactone.[3] Our investigations on the methyl ether, which appear to be somewhat more detailed than those for the parent dilactone and are reported here, confirm the postulated structure of the dilactone and uniquely prove the position of the picrotoxinin epoxide to be between carbon atoms 12 and 13.



(I)



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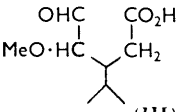

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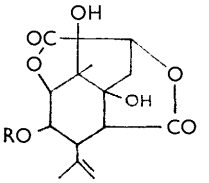


(IV)

The methyl ether of β-bromopicrotoxinic acid may be debrominated, to give the methyl ether of α-picrotoxinic acid (I; R = Me). Alkaline degradation of the dihydro-derivative would be expected to yield, on the basis of the Conroy structure, acids (II) and (III or the corresponding dicarboxylic acid). Acid (II) could be identified with certainty. This result is in accord with those previously obtained.[1] Methylation of β-bromopicrotoxinin directly with dimethyl sulphate gave, in varying yield, the easily crystallised methyl ether of β-bromopicrotoxinic acid, together with a water-soluble oil. Debromination of this intractable oil led to a neutral compound, $C_{16}H_{20}O_6$, m. p. 279°, which was unexpectedly alkali-stable. An alternative method of preparation, developed later, was by alkaline hydrolysis of the methyl ether of β-bromopicrotoxinic acid with subsequent debromination. Apopicrotoxinic dilactone was independently reported and formulated [3] as (IV; R = H) a little later, and from a comparison of infrared and chemical data it was evident that our derivative was probably the methyl ether of apopicrotoxinic dilactone (IV; R = Me). The method of preparation suggests that its formation is the result of alkaline hydrolysis rather than of a transformation during debromination, *i.e.*, analogous to the formation of the parent dilactone. The methyl ether as well as the acid [1] (V), m. p. 251°, are of particular interest, because they are derivatives of picrotoxinin in which the oxiran ring is present as a potential vicinal glycol unit; this unit, by its nature, facilitates a test of structure. (Other derivatives, in which the oxiran ring has been shown as open, but which led to sequestration of the 12-oxygen atom in ketal or acetal groups, [1,3,4] have not proved amenable to further investigation and confirmation.)

The methyl ether may be unambiguously placed at position 3. Infrared hydroxyl absorption (3458 and 3368 cm.$^{-1}$) in the methyl ether and in its monoacetate (3429 cm.$^{-1}$), which is readily formed on acetylation, necessitates at least two free hydroxyl groups in the methyl ether. They cannot be vicinal since the compound is unreactive to periodic acid, but one must be either activated or secondary. That the former is the correct

[1] Part IX, *J.*, 1959, 130.
[2] Slater, *J.*, 1949, 806.
[3] Conroy, *J. Amer. Chem. Soc.*, 1957, **79**, 1726.
[4] Burkhill, Holker, Robertson, and Taylor, *J.*, 1957, 4945.

alternative is demonstrated by formation of a dimethyl ether with diazomethane; this still shows hydroxyl absorption (3360 and 3494 cm.$^{-1}$). The neutral nature of the monomethyl ether eliminated the presence of a carboxyl group; the presence of two lactones (one a γ-, the other a δ-lactone) is required by the infrared spectrum (1806 and 1732 cm.$^{-1}$), varying slightly in the dimethyl ether (1771 and 1737 cm.$^{-1}$) and acetate (1793 and 1718 cm.$^{-1}$). The isopropenyl group is present, as deduced both from the spectrum (1653 cm.$^{-1}$) and from normal unsaturation tests. The seven oxygen atoms may therefore be assigned functions as two lactones, one methoxyl, and two non-vicinal hydroxyl groups.

The high frequency of the γ-lactone band of the monomethyl ether and its acetate suggests strongly that this is due to the 14-carbonyl group in the same lactone as in picrotoxinin * (1795 cm.$^{-1}$) and picrotoxic acid (1795 cm.$^{-1}$), the evidence for which in the latter case is certain. If this view is correct, the 15-carbonyl group may close on to position 12 or 13 in order to give a δ-lactone. The activated hydroxyl group must be at position 13, quite apart from possible steric considerations precluding closure, and hence the alternative is position 12. Such a hypothesis should be amenable to verification.

The incorporation of a molecule of water in picrotoxinin, whilst still maintaining a dilactonic structure, requires in the first instance, rupture of the ether linkage irrespective of the actual nature of such a system. It has been assumed above that the activated hydroxyl group is present at position 13 which corresponds to one end of the ether link in picrotoxinin.[3,5,6] Earlier work [1,3] has clearly shown that a hydroxyl group can arise at position 13 during reduction, and in picrotoxic acid [4] this hydroxyl group is present in the molecule. In our own case, reduction of the ether (IV; R = Me) with sodium borohydride yields a monolactonic (1728 cm.$^{-1}$) hemiacetal possessing one glycol unit. This last group can be formed only if, in this instance, there was a free hydroxyl group α to an original lactone, *i.e.*, at position 13. Hence closure of the 15-carbonyl group must be on to carbon 12. This argument in itself lends strong support for the presence of an oxygen function at position 12 not originally present in either of the picrotoxinin lactones. Absolute confirmation is provided from the following periodate studies.

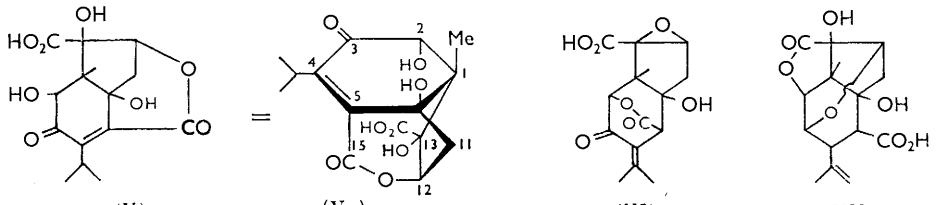
Neither apopicrotoxinic dilactone (IV; R = H) nor its methyl ether (IV; R = Me) reacts with periodic acid. After alkaline hydrolysis, however, when both lactones are open, uptake of sodium periodate is of the expected order; the rate of oxidation of apopicrotoxinic dilactone is about twice that of the monomethyl ether in which one potential glycol unit is removed by methylation of the 3-hydroxyl group. The position of this remaining glycol unit in the monomethyl ether was indicated by attempted periodate oxidation of the dimethyl ether. No oxidation occurred at an acid pH, but at an alkaline pH, and after hydrolysis of the two lactones, in contrast to the cases with the monomethyl ether and the parent dilactone, reaction with sodium metaperiodate was negligible; *i.e.*, in the ether (IV; R = Me) in which the 13-hydroxyl group is free, alkaline hydrolysis releases one vicinal glycol unit, but in the dimethyl ether, when the 13-hydroxyl group is sequestered in a methyl ether no potential vicinal glycol remains. Consequently, in apopicrotoxinic dilactone and its methyl ethers there must be a potential hydroxyl group at position 12 forming part of a lactone system in these compounds. Further, this must represent the second position of attachment of the ether system in picrotoxinin, thus providing unique confirmatory evidence, from a stepwise attack on the molecule, determining the exact nature of the ether ring in picrotoxinin as an epoxide falling between positions 12 and 13.

A feature of the chemical properties of apopicrotoxinic dilactone and its derivatives has been their stability in alkali. We have found this test useful as a convenient means

* It is reasonable to assume that the 6-hydroxyl group released by debromination has remained free in the resulting relactonisations. The axial conformation precludes any possible reaction with the 14-carbonyl group.

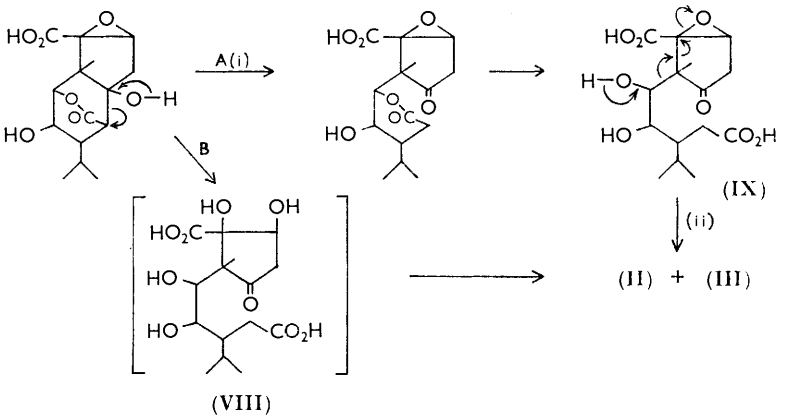
[5] Conroy, *J. Amer. Chem. Soc.*, 1951, **73**, 1889.
[6] Johns, Slater, and Woods, *J.*, 1956, 4715.

of distinguishing qualitatively between the mono-methyl ethers of α-picrotoxinic acid and apopicrotoxinic dilactone, and similarly between the acid of m. p. 251° (V), which is stable to alkali, and the acid of m. p. 219° (VI), which is degraded by hot alkali.[1] Comparison of the structures of compounds (IV; R = H, Me), (V), and (VII) suggests that previously held prerequisites for degradation require amending. In these compounds, the 6-hydroxyl group is free, a structural feature defined [7] much earlier and still holding without exception. Picrotoxinin, dihydropicrotoxinin, and picrotin titrate in cold alkali as dibasic substances,[8]



(V) (Va) (VI) (VII)

i.e., both lactone groups are opened but in the last two cases they are closed again on acidification. In warm alkali all three readily undergo degradation. These facts do not support the contention that the 15-carbonyl group must be present in a lactone for degradation to occur,[4] and indeed, compounds (IV; R = H, Me) and (V) do fulfil this requirement although they are alkali-stable. The stability of the lactone is desirable, if at all, for the first dealdolisation only [see (i) in scheme below]. This is clear from a consideration of the mechanism of degradation proposed by Conroy [3] for α-picrotoxinic acid, which adequately explains that of picrotoxinin also.



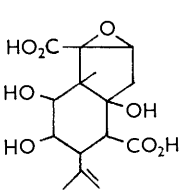
(VIII)

An elaboration of this mechanism (route B) by Burkhill *et al.*[4] in which compound (VIII) is considered an intermediate appears unlikely, because both the lactones (IV) and (V) in alkaline solution possess certain structural features similar to those in (VIII) but are stable under these conditions. The crucial point in our view is emphasized by a a comparison of the mechanistic path which it is suggested (VIII) must follow, compared with (IX), leading to (II) and (III). The development of a positive charge at position 13 is facilitated by the scission of the C–O bond made possible by the presence of a 12,13-epoxide. Such a scission would be expected, and provides a driving force for the concerted mechanism envisaged in the second dealdolisation (A, ii). Such motivation is not present and would not be expected from an intermediate of the nature of (VIII). We regard this as a sufficient explanation of the stability of lactones (IV) and (V) to alkali and for that

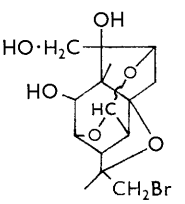
[7] Benstead, Gee, Johns, Martin-Smith, and Slater, *J.*, 1952, 2292.
[8] Benstead, Brewerton, Fletcher, Martin-Smith, Slater, and Wilson, *J.*, 1952, 1042.

of picrotoxic acid, although in the last case the steric rigidity enforced by the oxide ring and other steric factors as suggested by Conroy [9] probably play a major rôle. The sole exception to this generalisation is picrotoxinindicarboxylic acid. This compound has been formulated [9] as (X) and, whilst having an epoxide and free 2- and 6-hydroxyl groups, is still stable to hot alkali. There is at present remarkably little published chemistry of this derivative and we prefer to withold further comment at this stage.

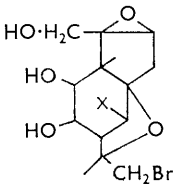
The structure of acid (V) and its formation from (VI) deserve further comment. The two-dimensional representation of (V) shows a formal resemblance to (IV). Compound (IV; R = H) is formed as a result of alkaline treatment of β-bromopicrotoxinic acid before debromination.[3] Similar treatment with alkali is necessary in the preparation of the ether (IV; R = Me). By contrast, formation of the acid (V) proceeds simply *via* the



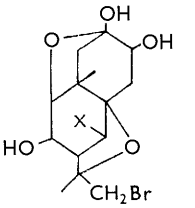
(X)



(XI)



(XII) X = CH_2·OH



(XIII) X = CH_2·OH

epoxide (VI) which is itself unstable to warm alkali. Debromination of methyl β-bromo-oxopicrotoxinate leads, in a manner analogous to the formation of the parent acids,[1] to the methyl esters of acids (V) and (VI) in a yield dependent upon the time of reaction. This result confirms the original formulations, particularly in so far as it indicates the 14-carbonyl group to be in the carboxyl group in the free acids.

The formation of acid (VI) by debromination of β-bromo-oxopicrotoxinic acid involves movement of a potential βγ-double bond into conjugation with the cyclic ketone, and is best regarded as an example of a " three-carbon " system, where the αβ-unsaturated form would be expected to predominate exclusively.[10] The further conversion (VI) ⟶ (V) is the result of interplay of several factors. Movement of the double bond to an endocyclic position can occur after, or more probably simultaneously with, isomerisation of the 15-carbonyl group to a δ-lactone closing at position 12 (this presumably occurs by rearward attack [3] of the carbonyl group on the epoxide ring). Such an isomerisation receives a driving force from the favoured movement of the double bond from an exo- to an endocyclic position.[11] This explains the ready formation of the lactone (V) under normal debromination conditions which leave the lactone in α-picrotoxinic acid unaffected. The probable conformations of acids (V) and (VI) are in harmony with their chemical reactivities. The conformation of (VI) will be little altered by the presence of the exocyclic double bond from that suggested by Conroy for α-picrotoxinic acid.[9] The closure of the 15-carbonyl group in a δ-lactone permits the four carbon atoms at positions 2, 3, 4, and 5 to assume more readily a planar or near-planar conformation and, in so doing, removes the isopropylidene group further from the axial 6-hydroxyl group. This explains the non-bromination of (VI) in contrast to neopicrotoxinin where the situation is more akin to that in picrotoxinin. If a model of (V) (cf. Va) is considered, the endocyclic double bond results in flattening of the six-membered ring and as a consequence the 2-hydroxyl group assumes a quasi-equatorial position sufficient to prevent relactonisation with the 14-carbonyl group. In our experience the acid (V) has shown no tendency to relactonise.

Part VIII [6] reported that reduction of β-bromopicrotoxinin by lithium aluminium

[9] Conroy, *J. Amer. Chem. Soc.*, 1957, **79**, 5550.
[10] Ingold, " Structure and Mechanism in Organic Chemistry," Bell and Sons Ltd., London, 1953, p. 562.
[11] Brown, Brewster, and Scheckter, *J. Amer. Chem. Soc.*, 1954, **76**, 467.

hydride gave two products, $C_{15}H_{21}O_6Br$ (XI) and $C_{15}H_{23}O_6Br$ (XII), the former in a very small yield, the latter in larger yield and in most cases the only product. The limited reactions reported for compound (XI) are not inconsistent with the formulation suggested by Conroy [12] as shown. The major product received formulation (XIII) in preference to the expected (XII) on the basis of a change (XII) ⟶ (XIII) under the conditions used in working up,[12] the reported stability to boiling 2N-mineral acid and diazomethane notwithstanding. We have repeated the reduction and employed sodium sulphate for hydrolysis of the reaction complex. The major product (XII) was obtained. This proved intractable in further transformations and because of this the reduction of chloropicrotoxinin [13] was investigated. The product, $C_{15}H_{23}O_6Cl$, showed only hydroxyl absorption in the infrared region. A triacetate was prepared readily and showed an infrared spectrum almost identical with that of the triacetate from compound (XII). Both acetates still showed hydroxyl absorption. Oxidation experiments with reduced chloropicrotoxinin were unpromising and in this respect completely paralleled those with the bromide (XII). Reaction with periodic acid, however, gave differing results. Whereas the bromo-compound (XII) absorbed 3 equivalents in acid media or one equivalent in neutral solution, reduced chloropicrotoxinin reacted with only one equivalent of periodic acid; no free chlorine could be detected although it had been expelled during reaction, as the end product was evidently halogen-free though intimately contaminated with halogenated material. Formaldehyde could not be detected in any of the periodate reaction mixtures. These reactions, whilst not inconsistent with structure (XII) or (XIII), were equally of no help in establishing one or other formulation. On re-investigation, further acetylation of the readily formed triacetate of compound (XII) gave a tetra-acetate showing no infrared hydroxyl absorption. Compound (XII) therefore contains only four hydroxyl groups, eliminating the possibility of an acetal-type structure of the nature of (XI). Efforts to distinguish further between the functions of the hydroxyl groups were unsuccessful; two are clearly present in a vicinal glycol as shown by uptake of one equivalent with lead tetra-acetate.[6] Periodic acid, as earlier reported,[6] leads to a complex reaction with eventual uptake of 3 mols. and no great emphasis can be placed on the quantitative value of this particular reaction. The formation of formaldehyde from hot oxidising solutions is, however, of much significance. From a solution containing unchanged periodate a 21% yield of formaldehyde was isolated as its 2,4-dinitrophenylhydrazone and colorimetric determination [14] indicated a 23% yield. If compound (XII) was boiled with hydrochloric acid before reaction with periodate, formaldehyde could be detected in a 36% yield. These results, we contend, are fully consistent with formulation (XII) rather than (XIII).

Experimental

Debromination of the Methyl Ether of β-Bromopicrotoxinic Acid.—The methyl ether (1 g.), in ethanol, was debrominated with zinc dust and ammonium chloride. Excess of zinc was filtered off, and the filtrate evaporated nearly to dryness under a vacuum. The addition of 2N-sulphuric acid (5 ml.) precipitated a white solid, which after 2 hr. was filtered off (0·5 g.) and crystallised from water. The *methyl ether* of α-picrotoxinic acid had m. p. 127—129° (Found: C, 60·0; H, 6·2; OMe, 10·2. $C_{16}H_{20}O_7$ requires C, 59·2; H, 6·2; 1OMe, 9·1%), $\nu_{max.}$ (in Nujol) 3548w, 3463w, 3358w, 1751w infl., 1731s, 1683s, 1650vw, and 1628w cm.$^{-1}$.

Methyl Ether of Dihydro-α-picrotoxinic Acid.—The methyl ether of α-picrotoxinic acid was hydrogentated in ethanol over platinum oxide. *Dihydro-α-picrotoxinic acid methyl ether*, crystallised from aqueous ethanol, had m. p. 214—215° (Found: C, 59·2; H, 6·9; OMe, 9·7. $C_{16}H_{22}O_7$ requires C, 59·3; H, 7·1; 1OMe, 9·5%), $\nu_{max.}$ (in Nujol) 3593w, 3483m, 1764w infl., 1746s, 1738s, 1689s, and 1634m cm.$^{-1}$.

Alkaline Degradation of the Methyl Ether of Dihydro-α-picrotoxinic Acid.—The finely powdered ether (3·0 g.) was dissolved in 2% aqueous potassium hydroxide (150 ml.) and stirred

[12] Conroy, *Chem. and Ind.*, 1957, 704.
[13] Meyer and Bruger, *Ber.*, 1898, **31**, 2958.
[14] Hough, Powell, and Woods, *J.*, 1956, 4799.

at 80° by a stream of nitrogen for 50 min. (Unchanged material only was isolated when 10% aqueous sodium carbonate was used.[15]) The red-brown alkaline solution was continuously ether-extracted, but yielded no product. The reaction solution was acidified to Congo Red and solid sodium carbonate was then added until the solution was just acid to litmus. Extraction with ether at this pH yielded a yellow oil (extract A) (0·9 g.). At pH 3 extract B (0·13 g.) was obtained, and on further acidification (Congo Red) with concentrated hydrochloric acid a resin (extract C) (2·9 g.) was obtained. Extract A gave no carbonyl or acid derivatives and, as with extract B, remained an intractable oil. Extract C showed $\lambda_{max.}$ 235 mμ (in ethanol), and with ethanolic 2,4-dinitrophenylhydrazine yielded a hydrazone, m. p. 219—220° (from ethanol). This showed no depression of m. p. when admixed with an authentic 2,4-dinitrophenylhydrazone, m. p. 220—222°, of the cyclic ketone (II). Descending chromatography on Whatman No. 1 paper of extract C, and acid (II), and development by spraying with litmus solution gave the following results:

Solvent	Extract C (R_F)	Compd. (II) (R_F)
PrOH–NH_3–H_2O, 80 : 4 : 16	0·59	0·59
EtOH–NH_3–H_2O ,, ...	0·62	0·62
Pyridine–NH_3–H_2O, 75 : 5 : 20	0·84	0·84

Methyl Ether of Apopicrotoxinic Dilactone.—(*a*) A solution of 40% aqueous potassium hydroxide (30 ml.) was added slowly to β-bromopicrotoxinin (4·8 g.) suspended in boiling water (10 ml.). When the solution had cooled to below 25°, dimethyl sulphate (5 ml.) was added dropwise with stirring. The solution was kept alkaline, and the temperature below 25°. The solution was acidified with concentrated hydrochloric acid and kept for 2 hr. at 0°, then inorganic material was filtered off. The filtrate was continuously extracted with ether, to yield a golden-yellow oil (3·73 g.). This oil (0·5 g.) was debrominated, in ethanol, with zinc dust and ammonium chloride. The residue, after removal of solvent, was acidified with 2N-sulphuric acid, precipitating the *apopicrotoxinic dilactone methyl ether* (0·28 g.), which recrystallised from aqueous acetone as needles, m. p. 279—281° (Found: C, 58·9; H, 6·3; OMe, 9·7. $C_{16}H_{20}O_7$ requires C, 59·3; H, 6·2; 1OMe, 9·6%), $\nu_{max.}$ (in Nujol) 3458s, 3368w, 1806s, 1732s, and 1653w cm.$^{-1}$. The dilactone decolorised bromine water, was recovered unchanged from 5N-sodium hydroxide, did not colour when heated in alkaline solution, and showed negligible reaction with neutral sodium metaperiodate. When the dilactone was boiled for 30 min. with 0·1N-sodium hydroxide and then allowed to react with an excess of sodium metaperiodate, the uptake after 4·6, 5·0, 8, 9, and 27 hr. amounted to 0·5, 0·84, 1·0, 1·57, and 3·01 equiv. respectively (apopicrotoxinic dilactone after similar treatment showed after 4 and 8 hr., uptake of 1·1 and 1·5 equivs. respectively).

(*b*) β-Bromopicrotoxinic acid methyl ether (1·1 g.) was dissolved in an aqueous solution of potassium hydroxide (0·69 g.) and kept at about 100° for 1 hr. To the boiling solution were added acetic acid (1·5 ml.), then ammonium chloride (0·54 g.) and zinc dust (1·6 g.) in small portions. The solution was boiled for a further 10 min. and filtered. The residue was washed with dilute acetic acid, and the combined filtrates were made up to 50 ml. with water and kept at 0°. The dilactone (IV; R = Me) (0·3 g.) crystallised (m. p. 279°). The *acetate*, prepared by acetic anhydride and pyridine, crystallised from ethanol in plates, m. p. 283—285° (Found: C, 59·0; H, 6·2. $C_{18}H_{22}O_8$ requires C, 59·1; H, 6·1%), $\nu_{max.}$ (in Nujol) 3429s, 1793s, 1745s, 1718s, and 1650w cm.$^{-1}$.

The Dimethyl Ether of (IV; R = Me).—The monomethyl ether (IV; R = Me) (0·28 g.), dissolved in methanol (25 ml.), was treated with diazomethane in ether until a yellow colour was maintained for 1 hr. The solvent was removed under a vacuum, ethyl acetate added to the residue, and unchanged material (0·05 g.) filtered off. The filtrate yielded *apopicrotoxinic dilactone dimethyl ether* (0·17 g.), m. p. 238—239° (from aqueous ethanol) (Found: C, 60·2; H, 6·4; OMe, 18·1. $C_{17}H_{22}O_7$ requires C, 60·3; H, 6·6; 2OMe, 18·3%), $\nu_{max.}$ (in Nujol) 3489m, 3360m, 1771s, 1737m, and 1639w cm.$^{-1}$. The dimethyl ether showed reaction neither with periodic acid nor, after pretreatment with alkali, with sodium metaperiodate.

Debromination of Methyl β-Bromo-oxopicrotoxinate.—(*a*) The ester (2·06 g.) was debrominated with zinc dust and ammonium chloride in ethanol, the total time of reaction being extended to 25 min. Removal of solvent, and acidification of the residue, gave a white solid (0·73 g.).

[15] Cf. Slater and Wilson, *Nature*, 1951, **167**, 324.

The *methyl ester* of (V), crystallised from water, had m. p. 121° (Found: C, 56·3; H, 6·2. $C_{16}H_{20}O_8$ requires C, 56·5; H, 5·9%), $\lambda_{max.}$ (in EtOH) 249 mμ (log ε 3·89), $\nu_{max.}$ (in Nujol), 3607s, 3438s, 3300s, 1740s, 1712s, 1682s, 1623s, and 1607s cm.$^{-1}$ [acid (V) shows $\lambda_{max.}$ 247 and 318 mμ (log ε 3·95 and 1·79 respectively), $\nu_{max.}$ (in Nujol) 3480m, 3410m, 3370m, 1749s, 1718m, 1678m, 1629vw, and 1598m cm.$^{-1}$]. The ester decolorises bromine-water immediately and gives a positive Baeyer test. Tollens's reagent is reduced in the cold, but no colour is obtained by heating the acid with 2N-alkali. The filtrate, after acidification, was continuously extracted with ether, yielding an intractable oil.

(*b*) The oxo-ester (2·2 g.) was debrominated as above, but the time of reaction was no longer than 15 min. After removal of solvent and addition of 2N-sulphuric acid, the solution was kept at 0° for 2 days; product (0·65 g.) crystallised. Recrystallised from ethanol, the compound had m. p. 178° alone or mixed with a sublimed sample of the methyl ester [1] prepared from acid (VI), and had $\lambda_{max.}$ (in EtOH) 261 and 331 mμ (log ε 4·05 and 1·72 respectively). The original filtrate was continuously extracted with ether, and the resultant oil was chromatographed in ether on a silica gel column, separating it into an intractable oil, and solid material, m. p. 121° (from water), identical with the ester obtained in (*a*).

Reduction of β-Chloropicrotoxinin with Lithium Aluminium Hydride.—β-Chloropicrotoxinin (5 g.) in dry dioxan (50 ml.) was added dropwise to a stirred suspension of lithium aluminium hydride (2 g.) in dry ether (80 ml.), and the suspension was refluxed for 45 min. Ethyl acetate (5 ml.) was added to the cooled reaction mixture, followed by a saturated aqueous solution of sodium sulphate (20 ml.). Solvent was removed under a vacuum, and the residue extracted with boiling absolute ethanol (5 × 100 ml.). When the alcohol had been removed, the residual brown gum was extracted with dry ether in a Bolton extractor, giving needles (2 g.) of a *compound* which, sublimed at 165°/0·02 mm., had m. p. 218° (Found: C, 54·2; H, 6·6; Cl, 10·8. $C_{15}H_{23}O_6Cl$ requires C, 53·8; H, 6·9; Cl, 10·6%), $\nu_{max.}$ (in Nujol) 3353m, 3278m, and 1415vw cm.$^{-1}$. The *triacetate*, prepared by acetic anhydride and pyridine, was distilled to a glass (150°/0·05 mm.), m. p. 74—78° (Found: C, 54·4; H, 6·2; Cl, 6·5; Ac, 30·2. $C_{21}H_{29}O_9Cl$ requires C, 54·7; H, 6·3; Cl, 7·7; 3Ac, 28·0%), $\nu_{max.}$ (in Nujol) 3620w, 3490w, 1742s, and 1639w cm.$^{-1}$.

Triacetate of Reduced β-Bromopicrotoxinin.—Reduced β-bromopicrotoxinin was kept in pyridine and acetic anhydride at room temperature overnight. After removal of solvent, the residual oil was distilled (150—160°/0·05 mm.) to a glass. The *triacetate* had m. p. 90—92° (much previous softening from 82°) (Found: C, 50·0; H, 5·7; Br, 15·9. $C_{21}H_{29}O_9Br$ requires C, 49·9; H, 5·7; Br, 15·8%), $\nu_{max.}$ (in Nujol) 3525m and 1748s cm.$^{-1}$.

Tetra-acetate.—The preceding triacetate, m. p. 90—92° (0·4 g.), was refluxed with acetic anhydride (15 ml.) and sodium acetate (0·3 g.) for 14 hr. during which it darkened. Water was then added, and the solution extracted with ether. The extract was taken to dryness and the residual oil distilled. The *tetra-acetate* had m. p. 82° (previous softening) (Found: C, 50·7; H, 5·6; Br, 14·3; Ac, 29·5. $C_{23}H_{31}O_{10}Br$ requires C, 50·5; H, 5·7; Br, 14·6; 4Ac, 31·4%), $\nu_{max.}$ (in Nujol) 1737 cm.$^{-1}$.

Periodate Oxidation of Reduced β-Bromopicrotoxinin.—(*a*) A solution of reduced β-bromopicrotoxinin (0·1 g.) in 0·5M-periodic acid (3 ml.) was kept overnight at room temperature. Water (20 ml.) was added, and the solution distilled into a solution of 2,4-dinitrophenylhydrazine (20 ml. of saturated solution in N-sulphuric acid). The precipitate of formaldehyde hydrazone was filtered off (0·21 mol.); m. p. and mixed m. p. 158—160°. If the formaldehyde was determined colorimetrically [14] before distillation, a yield of 0·23 mol. was calculated.

(*b*) Reduced β-bromopicrotoxinin (0·3 g.) was refluxed with concentrated hydrochloric acid (4 ml.) for 30 min. Water was added, and all mineral acid removed as azeotrope. 0·5M-Periodic acid (5 ml.) was then allowed to react; after 30 min. chlorine was detected. After 7 hr. at room temperature formaldehyde was determined,[14] a 36% yield being recorded.

Reduction of (IV; R = Me) *by Sodium Borohydride.*—Sodium borohydride (1·0 g.) in water (10 ml.) was added slowly to a solution, cooled in ice, of the monomethyl ether (0·4 g.) of apopicrotoxinic dilactone in dioxan (25 ml.) and water (10 ml.). The solution was kept at room temperature for 24 hr. with occasional further additions of small amounts of sodium borohydride in water. Any insoluble material was then filtered off, and the filtrate reduced to about 10 ml., acidified to Congo Red, and kept at 0° overnight. Boric acid crystallised. This was filtered off, and the filtrate continuously extracted with ether. The extract, after removal of ether, was distilled several times with methanol, and the *monolactone* (0·36 g.)

recrystallised from water as needles, m. p. 236° (Found: C, 57·7, 57·7, 57·6; H, 6·7, 7·2, 7·0; OMe, 9·8. $C_{16}H_{22}O_7,\frac{1}{2}H_2O$ requires C, 57·3; H, 6·9; 1OMe, 9·3%), $\nu_{max.}$ (in Nujol) 3412s, 3366s, 1728s, and 1639w cm.$^{-1}$. The compound reduced Tollens's reagent on warming. Periodic acid uptake was 0·82 and 0·96 equiv. in 5 and 29 hr. respectively.

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