922. *Picrotoxin and Tutin. Part VIII.**

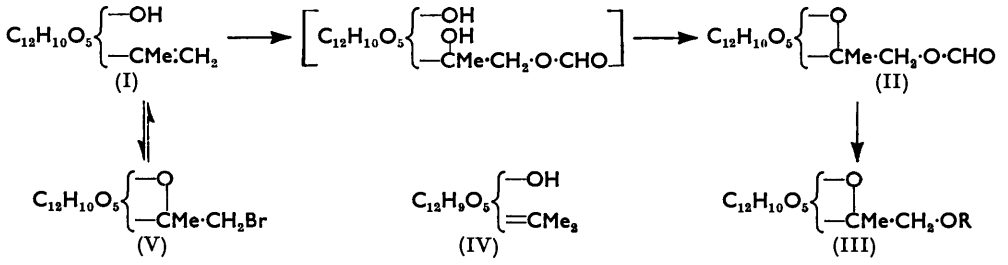
By R. B. Johns, S. N. Slater, and R. J. Woods (with, in part, D. Brasch and Roy Gee).

Hydroxylation of the double bond of picrotoxinin has been studied and it is shown that with performic acid a product is obtained closely analogous to bromopicrotoxinin. Acylation and active-hydrogen studies are described. Reduction of picrotoxinin and several of its derivatives with lithium aluminium hydride is described, together with the action of periodic acid on the reduction products. The resulting evidence for a hemiacetal structure leads to tentative formulation of picrotoxinin as either (XX) or (XXI).

In Part IV [1] the functional groups of the picrotoxin–tutin series of compounds were discussed. Further experiments bearing on this problem are now described.

THE DOUBLE BOND

Halogenation of picrotoxinin and tutin, giving saturated monohalogeno-compounds, is one of the most characteristic reactions in this series. It is closely paralleled by the hydroxylation of picrotoxinin (I) with performic acid to give hydroxypicrotoxinin formate (II), $C_{16}H_{16}O_8$, a saturated non-reducing compound hydrolysed by formic acid to

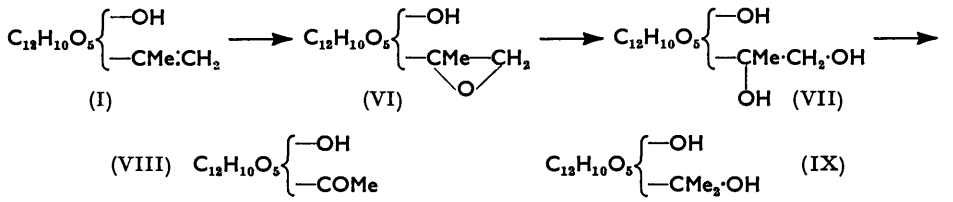


hydroxypicrotoxinin (III; R = H) which is stable to periodic acid and to lead tetra-acetate. Bromopicrotoxinin and hydroxypicrotoxinin are best formulated with the oxide ring on the tertiary rather than the primary carbon of the *iso*propyl group for the following reasons: (*a*) both picrotoxinin and *neo*picrotoxinin (IV) undergo bromination

* Part VII, *J.*, 1954, 1953.

[1] Benstead, Brewerton, Fletcher, Martin-Smith, Slater, and Wilson, *J.*, 1952, 1042.

in a similar fashion and it seems likely that the oxide ring is formed on the "unsaturated" carbon common to the two compounds; (*b*) hydroxypicrotoxinin readily forms a monoacetate (III; R = Ac) and gives a positive response to chromic acid in acetic acid, suggesting that it is a primary rather than a tertiary alcohol. The tertiary carbon atom of bromopicrotoxinin (V) is asymmetric and two isomerides are known. By contrast, hydroxypicrotoxinin formate was isolated in one form only, possibly because the entering group has a different steric requirement.



With perbenzoic acid, picrotoxinin gave an epoxide (VI), readily hydrolysed to dihydroxypicrotoxinin (VII) which is converted by periodic acid into α-picrotoxinone (VIII) and formaldehyde, which confirms the presence of a terminal methylene group. The stability of dihydroxypicrotoxinin is unexpected, since its formulation as (VII) suggests ready loss of water to give hydroxypicrotoxinin (III; R = H). This stability is presumably due to a sterically unfavourable configuration of the tertiary hydroxyl group. Although perbenzoic and performic acid give different products from picrotoxinin, no such difference is observed in the *neo*-series where, *e.g.*, acetyl*neo*picrotoxinin yields, with either reagent, acetylhydroxy*neo*picrotoxinin which according to its analysis and stability to periodic acid and to lead tetra-acetate lacks the α-glycol system. The oxide (VI) is a possible intermediate in conversion of picrotoxinin into picrotin (or an isomer) (IX) in which the presence of a dimethylcarbinol group seems established.

The presence in *neo*picrotoxinin (IV) of an *iso*propylidene group attached to a disubstituted carbon atom is confirmed by the ultraviolet absorption spectrum, which shows relatively strong absorption in the region 215—220 mμ (ε 3140 at 215 mμ) characteristic of tetrasubstituted double bonds.[2] No other derivative of picrotoxin showed strong absorption in this region.

Hydroxypicrotoxinin resembles bromopicrotoxinin in giving a hydroxy-acid (hydroxypicrotoxinic acid), $C_{15}H_{18}O_8$, with dilute aqueous sodium hydroxide. The same acid was obtained by treating α-picrotoxinic acid with perbenzoic acid, but performic acid yielded an isomeric acid. The relation between the two may be similar to that between α- and β-bromopicrotoxinic acid.

Meyer and Bruger [3] have described iodopicrotoxinin. We have been unable to repeat this preparation and have failed in attempts by alternative methods. The melting point of Meyer and Bruger's product (198—199°) is practically identical with that of their picrotoxinin (200—201°) and no mixed melting point is recorded. We believe that the so-called iodopicrotoxinin is in fact merely recovered picrotoxinin.

The above reactions, and the properties of the substances described, confirm certain generalisations previously advanced but reveal some unexpected irregularities. Consistently with the conclusion by Benstead *et al.*[4] that formation of the oxide ring of the bromo-compounds sequesters the hydroxyl group essential for alkali-fission and consequent generation of reducing substances, the hydroxylation products formulated with an oxide ring are non-reducing. Dihydroxypicrotoxinin resembles dihydropicrotoxinin in that, unlike picrotoxinin, it can be recovered from solution in cold dilute aqueous sodium hydroxide. Diazomethane has been postulated [4] as a reagent for distinguishing compounds of the "picrotin" series from those of the "picrotoxinin" series. This assumption is

[2] Bladon, Henbest, and Wood, *Chem. and Ind.*, 1951, 866; Halsall, *ibid.*, p. 867.
[3] Meyer and Bruger, *Ber.*, 1898, **31**, 2958.
[4] Benstead, Gee, Johns, Martin-Smith, and Slater, *J.*, 1952, 2292.

unjustified, since neither hydroxypicrotoxinin nor dihydroxypicrotoxinin is affected by diazomethane, and the behaviour of the picrotoxin series of compounds towards diazomethane remains therefore unpredictable and largely uncorrelated.

The Hydroxyl Groups (with D. BRASCH and ROY GEE).—Because the functions of the non-lactonic oxygen atoms of picrotoxinin and picrotin are still incompletely defined we have made certain acylation and active-hydrogen studies. These help to define the number of hydroxyl groups present, although acylation can give only minimum numbers and active-hydrogen results give maximum numbers—particularly in complex lactonic molecules it is difficult to decide whether there are groups other than hydroxyl which can provide active hydrogen atoms.

There are numerous references to acylation but the results are confused partly because early workers started with picrotoxin, and partly owing to the absence of full analytical details.

Paterno and Oglialoro [5,6] heated picrotoxin with acetic anhydride and anhydrous sodium acetate to obtain a small amount of a substance, m. p. 227°, formulated as "picrotin acetic anhydride," $C_{19}H_{22}O_9$, and larger amounts of a substance, m. p. 245°, which reacted with bromine and was formulated as "picrotoxinin acetic anhydride," $C_{19}H_{22}O_8$. Meyer and Bruger [3] state that the acetylation of picrotoxinin itself with either acetic anhydride and sodium acetate or boiling acetyl chloride yields a picrotoxinin diacetate, $C_{19}H_{20}O_8$, m. p. 254—255°, believed identical with Paterno and Oglialoro's material of m. p. 245°. We have been unable to confirm the acetylation of picrotoxinin with boiling acetyl chloride, the starting material being recovered after about 30 minutes' reaction. No better success was achieved with cold acetic anhydride and perchloric acid, or with acetyl chloride and magnesium (Spassow's method [7]). Sielisch [8] described the acetylation of bromopicrotoxinin with acetyl chloride or acetic anhydride to a monoacetate, m. p. 268°. We have been unable to confirm either of these acetylations, and also failed with anhydride–perchloric acid and Spassow's method, bromopicrotoxinin being recovered unchanged. As already described, hydroxypicrotoxinin yields a monoacetate (III; R = Ac).

Paterno and Oglialoro [9] state that picrotin and boiling acetyl chloride give a mixture of picrotoxide and a substance, m. p. 202°, formulated initially as a diacetate, $C_{19}H_{22}O_9$, but later [5] as a monoacetate, $C_{17}H_{20}O_8$. Meyer and Bruger,[3] by treating picrotin first with cold and then with boiling acetyl chloride, obtained a mixture of anhydrodiacetylpicrotin, m. p. 300°, and a substance, m. p. 207—210° believed identical with Paterno and Oglialoro's material of m. p. 202°, but for which a diacetate formula was preferred; no acetyl determination was reported. Meyer and Bruger also studied the reaction between picrotin and acetic anhydride–anhydrous sodium acetate, isolating a substance, m. p. 244—245°, believed identical with the substance, m. p. 227°, obtained by Paterno and Oglialoro from picrotoxin (see above). On the basis of elementary analysis and acetyl figures, however, it was formulated as a monoacetate, $C_{17}H_{20}O_8$. We have examined the acetylation of picrotin under different conditions. First, we confirm the formation of the monoacetate, $C_{17}H_{20}O_8$, m. p. 242—243°, when picrotin reacts with acetic anhydride–anhydrous sodium acetate; also that of a diacetate, $C_{19}H_{22}O_9$, m. p. 200°. We have, however, worked with acetic anhydride rather than acetyl chloride and find the reaction complex. In the cold, without a catalyst, no reaction occurred. In the presence of a trace of sulphuric acid a mixture was obtained and separated into (*a*) acetyl*neo*picrotoxinin, (*b*) an isomeric acetate, $C_{17}H_{18}O_7$, m. p. 245°, and (*c*) high-melting material regarded as either anhydropicrotin (see above) or its acetate [3] but not further investigated. When anhydrous ferric chloride was used as catalyst several products were also isolated: at room temperature after five days the diacetate, m. p. 200°, was readily obtained; when the reaction mixture was heated the main product was anhydropicrotin; a systematic fractionation of the product obtained after the reaction mixture had been kept for two days yielded

[5] Paterno and Oglialoro, *Gazzetta*, 1879, **9**, 57.
[6] *Idem, ibid.*, 1881, **11**, 36.
[7] Spassow, *Ber.*, 1937, **70**, 1925.
[8] Sielisch, *Ber.*, 1912, **45**, 2555.
[9] Paterno and Oglialoro, *Gazzetta*, 1877, **7**, 193.

(i) a small amount of acetyl*neo*picrotoxinin, (ii) a somewhat greater amount of material regarded as the impure isomeric monoacetate, and (iii) mainly the diacetate.

Paterno and Oglialoro state that picrotin and benzoyl chloride give a monobenzoate, $C_{22}H_{22}O_5$, m. p. 230°, and Schmidt [10] describes a (mono)benzoylpicrotin, m. p. 245°. Meyer and Bruger [3] describe both a monobenzoylpicrotin, m. p. 236°, believed identical with Paterno and Oglialoro's material, and a dibenzoate, $C_{29}H_{26}O_9$, m. p. 247°, which was obtained at higher temperatures. The only benzoate we have succeeded in preparing is the monobenzoate, m. p. 236°.

From the above we conclude that picrotoxinin contains no acylatable group but that picrotin may form either a mono- or a di-acetate, and that under the influence of the acetylating agent used it may undergo dehydration to either *neo*picrotoxinin or an isomer not hitherto described but now isolated as its acetate. The existence of such an isomer had been earlier suspected.[11] The infrared spectrum of the acetate shows carbonyl absorption at 1732 and 1790 cm.$^{-1}$ and double-bond absorption at 1649 cm.$^{-1}$ (identical with that of picrotoxinin and tutin), suggesting that it differs from *neo*picrotoxinin in possessing an *iso*propenyl group. Its chemistry has not yet been investigated.

The acetylation of dihydroxypicrotoxinin (VII) is interesting. On the basis of the non-acetylation of picrotoxinin and the presence of only one additional (primary) hydroxyl group capable of ready acetylation, it would be expected to form a monoacetate. In fact, it forms a triacetate and thus behaves in a manner analogous to that of dihydropicrotoxinin, which (unexpectedly) gives a diacetate.[1]

Attempts have been made, particularly by Horrmann,[12] to determine the number of hydroxyl groups by the Zerewitinoff method. The numbers do not lie very close to whole numbers and Mercer and Robertson [13] attribute a value such as 1·5 for picrotoxinin to tenaciously held water of crystallisation. As we have never experienced difficulty in drying specimens for analysis it seemed to us more likely that the figure 1·5 was low rather than high, and due to incomplete reaction between picrotoxinin and the Grignard reagent under the conditions commonly used at that time (room temperature). Modern analytical practice requires heating of the reaction mixture.[14] We have therefore redetermined the active hydrogen content of picrotoxinin and related substances, using an apparatus essentially similar to that of Hochstein.[15] Table 1 shows the results, and also Horrmann's figures, from which it is reasonable to assume that Horrmann did, in fact, work at room

TABLE 1.

Substance	Active hydrogen by Grignard reaction			Active hydrogen by lithium aluminium hydride	
	Horrmann	at 30°	at 98°	at 30°	at 98°
Picrotoxinin	1·5	1·45, 1·48	2·12, 2·08	1·8	2·2
β-Bromopicrotoxinin	0·3	0·32, 0·32	0·93, 0·96	0·8	1·1
Reduced β-bromopicrotoxinin	—	—	4·90, 4·72	—	
β-Bromopicrotoxinic acid	2·46	—	—	—	—
Methyl β-bromopicrotoxinate	—	1·80, 1·85	2·07, 2·10	1·8	2·1
Dihydropicrotoxinin	—	3·90, 3·90, 3·91	3·96, 3·95, 4·12	3·8	3·8
Dihydropicrotoxinin diacetate	—	—	4·02, 4·04	4·1	4·4
*neo*Picrotoxinin	—	2·91, 3·22	2·91, 3·22	3·2	3·3
Picrotin	2·1, 2·0	2·02, 2·00, 2·10	2·02, 2·00, 2·12	2·05	2·2
Acetylpicrotin	—	0·10, 0·10	0·12, 0·20	0·35	0·42
Benzoylpicrotin	—	1·51, 1·50	2·11, 2·06	1·8	2·2

temperature and that our figures at 98° represent completion of the initial reaction. The effect is particularly noticeable, for example, with β-bromopicrotoxinin. Lithium aluminium hydride is a useful alternative reagent for determining active hydrogen [16] and the Table gives also the figures obtained therewith.

[10] Schmidt, *Annalen*, 1883, **222**, 313.
[11] Slater and Wilson, *Nature*, 1951, **167**, 324.
[12] Horrmann, *Annalen*, 1916, **411**, 273.
[13] Mercer and Robertson, *J.*, 1936, 288.
[14] Pregl–Roth, ed. Grant, "Quantitative Organic Micro Analysis," Churchill, London, 4th edn., 1945, p. 134.
[15] Hochstein, *J. Amer. Chem. Soc.*, 1948, **70**, 305.
[16] Roger Adams, "Organic Reactions," Wiley, New York, 1951, Vol. VI, p. 469.

It seems clear that picrotoxinin contains two active hydrogen atoms and that one of these takes part in the bromination. If active hydrogen is equated with hydroxyl groups this is in accordance with views previously expressed.[4] The value of four active hydrogen atoms for dihydropicrotoxinin is unexpected. Hydrogenation of a double bond could conceivably remove an activating group and thus lower the figure, but would not be expected to raise it. An important difference has been observed, also, in the infrared spectra of the two substances—picrotoxinin absorbs at only one position in the 3μ region (at 3455 cm.$^{-1}$, in Nujol) whereas dihydropicrotoxinin has two bands (3527 and 3447 cm.$^{-1}$, in Nujol). The last bands are absent from the spectrum of diacetyldihydropicrotoxinin, which points to this substance's being a normal *O*-acetate and to the presence of two hydroxyl groups in dihydropicrotoxinin, as previously inferred. The fact that the diacetate still possesses four active hydrogen atoms may be due to the known activating influence of acetate groups on the hydrogen attached to adjacent carbon atoms.[17]

Picrotin shows only two active hydrogen atoms. This was unexpected, in view of the generally assumed relation between picrotin and picrotoxinin, and was at first attributed to incomplete reaction. In the Grignard reaction, however, no significant increase was obtained when a large volume of solvent was used (to decrease precipitation effects) and the reaction time was doubled. Again, *neo*picrotoxinin, the dehydration product of picrotin, shows an increase in its content of active hydrogen atoms over the latter, and acetyl- and benzoyl-picrotin give puzzling figures.

There is a clear conflict of evidence on the function of the sixth oxygen atom of picrotoxinin, which may be present in an ether or a hydroxyl group. The evidence in favour of two hydroxyl groups in dihydropicrotoxinin is very strong, and this, coupled with the active-hydrogen figures for picrotoxinin and β-bromopicrotoxinin and the lithium aluminium hydrogen reduction product of the latter (see below), suggests that picrotoxinin is a dihydroxy-compound. Against this is the somewhat uncertain relation between picrotoxinin and dihydropicrotoxinin.

If the sixth oxygen atom is part of a hydroxyl group, there must be either a third carbocyclic ring or a second double bond in picrotoxinin. The spectral evidence is against a second double bond. On the other hand the reaction between α-picrotoxinone and methanolic diazomethane leads to a compound, $C_{16}H_{18}O_7N_2$, corresponding to methylation of a hydroxyl group and addition of a second molecule of diazomethane, such as would be expected in the conversion of an olefin into a pyrazoline. A preliminary study of this compound by Mr. Ghulam Hassan has shown, however, that the infrared spectrum lacks absorption in the region expected for a C=N link, and the very ready loss of elementary nitrogen, either at the melting point or with cold mineral acid, also makes a pyrazoline structure unlikely. It may well be an intermediate of the type observed in reactions between aldehydes and diazomethane.[18] It is concluded that a second double bond is absent. There is no available evidence which bears closely on the question of a third carbocyclic ring.

No positive evidence for the presence of an ether linkage in picrotoxinin has hitherto been available, but strong experimental support is given below for the presence of a hemiacetal ether linkage, and in view of this it must be concluded that the balance of evidence is against a dihydroxy-structure for picrotoxinin. No explanation can be given for the anomalous behaviour of dihydropicrotoxinin.

The Lactone Groups and Centres of Acidity.—The high oxygen content of picrotoxinin and of picrotin suggests that lithium aluminium hydride reduction of these substances (or suitable derivatives) may produce polyhydric alcohols susceptible to attack by glycol-splitting reagents. Neither type of reaction is likely to proceed with isomerisation (such as accompanies all previously recorded degradations) and this offers hope of controlled stepwise degradation.

Reduction of β-bromopicrotoxinin, $C_{15}H_{15}O_6Br$, with lithium aluminium hydride gives (mainly) a crystalline alcohol, $C_{15}H_{23}O_6Br$, in which the increment of total hydrogen

[17] Fieser and Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, 3rd edn., 1949, p. 521.

[18] Eistert, in "Newer Methods of Preparative Organic Chemistry," Interscience Publ. Inc., New York, 1948, p. 513.

and in active hydrogen corresponds to the complete reduction of two lactone groups. The product was stable to boiling 2N-sulphuric acid, to boiling 0·5N-sodium hydroxide, and to boiling acidified silver nitrate solution. A smaller quantity of a second crystalline reduction product, $C_{15}H_{21}O_6Br$, was also isolated. Since the only isomerisation likely to occur during the reduction is one analogous to that effected in passing from bromopicrotoxinin to bromopicrotoxinic acid, and reduction of the latter (see below) gives entirely different products, we assume that no such isomerisation has occurred. Debromination of the major reduction product has given small quantities of two crystalline products.

Reduction of picrotoxinin itself, $C_{15}H_{16}O_6$, gives somewhat intractable syrups, but a small quantity of crystalline material has been isolated. It is formulated as $C_{15}H_{20}O_6$, suggesting that only one of the two lactone groups has been reduced, but there is virtually no characteristic infrared absorption in the carbonyl region, and the substance is unaffected by methanolic sodium methoxide which would be expected to open any residual lactone ring [there is an extremely faint band at 1792 cm.$^{-1}$ (in Nujol) which we attribute to slight contamination with incompletely reduced material, but no corresponding absorption with the dihydro-derivative or the methyl ether, described below]. The alternative interpretation is partial reduction of both lactone groups.[19] The compound is non-reducing but may be degraded with alkali. It yields a methyl ether, $C_{16}H_{22}O_6$, with both diazomethane and methyl sulphate, and is smoothly hydrogenated to a derivative, $C_{15}H_{22}O_6$.

Reduction of either β-bromopicrotoxinic acid or its methyl ester gives in high yield a crystalline product, probably $C_{15}H_{21}O_6Br$. Its infrared spectrum shows medium absorption at 1639 cm.$^{-1}$ (in Nujol), whose origin is not apparent. Attempted debromination of the reduction product gave no useful material.

The actions of periodic acid and of lead tetra-acetate on the above reduction products have been closely studied but some unexpected difficulties have been encountered. The reaction with reduced β-bromopicrotoxinic acid appears most straightforward : Table 2

TABLE 2. *Periodic acid oxidation of reduced β-bromopicrotoxinic acid.*

Time (hr.)	35 min.	2	6	24	46
Reagent reacted (atoms of O/mol.)	1·05	1·1	1·15	1·35	1·55
Acid formed (equiv./mol.)	0	0	0	0·1	0·2

shows these results. The crystalline product, $C_{14}H_{17}O_5Br$, obtained in 95% yield, is accompanied by some formaldehyde. It has also been obtained by the action of potassium permanganate. It has an ultraviolet maximum at 302—305 mμ, strong carbonyl absorption at 1768 cm.$^{-1}$, and gives a 2 : 4-dinitrophenylhydrazone. It is therefore formulated as a five-membered cyclic ketone.[20] The expected alcohol, $C_{14}H_{19}O_5Br$, obtained by reducing the ketone with lithium aluminium hydride, is not attacked by periodic acid.

If β-bromopicrotoxinic acid is formulated as (X), these changes may be represented as follows, in which the carbonyl group is regarded as present in the five-membered ring which appears in a number of degradation products of picrotoxin :

$$\underset{(X)}{C_{13}H_{16}O_4Br}\left\{>C(OH)\cdot CO_2-\xrightarrow{LiAlH_4} C_{13}H_{17}O_4Br\left\{>C(OH)\cdot CH_2\cdot OH\xrightarrow{HIO_4}\right.\right.$$

$$C_{13}H_{17}O_4Br\left\{>CO\xrightarrow{LiAlH_4} C_{13}H_{17}O_4Br\left\{>CH\cdot OH\right.\right.$$

The action of periodic acid on reduced β-bromopicrotoxinin, $C_{15}H_{23}O_6Br$, is complex and appears to take place in two stages. The first (rapid) reaction consumes one mol. of reagent and is followed by slower consumption of periodate accompanied by expulsion of free bromine. Table 3 summarises the quantitative aspects of the reaction. Bromine appears in the reaction mixture after about three hours.

If the oxidation is carried out with periodate in neutral or alkaline solution, or with lead tetra-acetate in glacial acetic acid, only one mol. of reagent is consumed (see Table 3)

[19] Arth, *J. Amer. Chem. Soc.*, 1953, **75**, 2413; Hinder and Stoll, *Helv. Chim. Acta*, 1954, **37**, 1866.
[20] Bellamy, " The Infra-red Spectra of Complex Molecules," Methuen, London, 1954, p. 114.

but the further action of acidic oxidising agents (periodic acid, iodic acid, acidified hydrogen peroxide) leads again to the expulsion of free bromine. Unfortunately no crystalline products could be isolated from the intermediate stage of oxidation, although two crystalline bromine-free end products, $C_{15}H_{22}O_7$ (?) and $C_{15}H_{24}O_6$ (?), have been obtained. Neither formaldehyde nor any other simple aldehyde or ketone has been regularly detected amongst the oxidation products (formaldehyde was identified once only). An important feature of Table 3 is that consumption of one mol. of periodic acid produces, from the

TABLE 3. *Oxidation of reduced β-bromopicrotoxinin*, $C_{15}H_{23}O_6Br$.

No.	Reagent	Time	Minutes 5	10	15	25	30	35	Hours 1	1·5
1	Periodic acid (acid solution)	Reagent reacted (atoms of O/mol.)	—	—	—	—	—	1·05	1·05	—
		Acid formed (equiv./mol.)	—	—	—	—	—	—	1·15	—
2	,,	$[\alpha]_D$ (assuming constant mol. wt.)	—	−86°	−76°	—	—	—	—	−76°
3	Periodate (alkaline solution [a])	Reagent reacting (atoms of O/mol.)	—	—	—	—	0·9	—	0·95	—
		Acid formed (equiv./mol.)	—	—	—	—	1·05 [b]	—	1·0 [b]	—
			—	—	—	—	1·45 [b]	—	1·35 [b]	—
4	Lead tetra-acetate	Reagent reacted (mol./mol.)	1·0	—	1·0	0·95	—	—	0·95	—

No.	Hours 2	3	5	7	7·5	18	21	23	24	45	47	48	72	90	116
1	1·0	—	—	1·4	—	—	—	2·1	—	—	2·95	—	—	—	—
	0·95	—	—	0·86	—	—	—	0·75	—	—	0·8	—	—	—	—
2	—	−76°	—	—	−66°	−60°	—	—	−60°	—	—	−48°	−49°	−47°	−46°
3	—	—	—	—	—	—	—	—	0·95	—	0·95	(2·0 [c])	—	—	—
	—	—	1·0 [b]	—	—	—	1·05 [b]	—	—	1·05 [b]	—	—	—	—	—
	—	—	1·05 [b]	—	—	—	0·9 [b]	—	—	0·9 [b]	—	—	—	—	—
4	0·95	—	—	—	—	—	—	—	1·1	—	—	1·2	—	—	—

[a] The oxidising medium was just alkaline to phenolphthalein. [b] A series of determinations in which the periodate reacting and the acid formed were determined simultaneously. [c] A portion of the solution was removed and acidified after 24 hr. and the periodate reacting determined after a further 23 hr.

completely neutral starting material, one equivalent of (back-titratable, *i.e.*, lactonic) acidity. This requires, in reduced bromopicrotoxinin, the grouping $-C(OH)\cdot\underset{\displaystyle C}{\underset{|}{C}}(OH)\cdot O-$ which on periodate cleavage would generate lactone and carbonyl functions. It is significant that the formula $C_{15}H_{22}O_7$ may be derived from $C_{15}H_{23}O_6Br$ by the two formal operations, (*a*) periodate cleavage, *i.e.*, loss of 2H, and (*b*) replacement of bromine by hydroxyl. Conductometric titration of the compound $C_{15}H_{22}O_7$ confirms the presence of one centre of acidity and the infrared spectrum shows very strong γ-lactone-carbonyl absorption at 1759 cm.$^{-1}$ (in Nujol). There is no other carbonyl absorption and any ketonic function (the compound is non-reducing) must be present in, *e.g.*, hemiacetal form. The above considerations suggest the partial formulations of bromopicrotoxinin (and by inference picrotoxinin) as (XI), reduced bromopicrotoxinin as (XII), and the compound $C_{15}H_{22}O_7$ as (XIII, modified by subsequent hemiacetal formation). The postulated hemiacetal-lactone system (XI) has implications on the lactonic behaviour and spectra of picrotoxinin and bromopicrotoxinin, which are discussed below.

The second periodate oxidation product, $C_{15}H_{24}O_6$, appears from its infrared spectrum [medium absorption at 1712 cm.$^{-1}$ (in Nujol)] coupled with its non-reducing properties, to be a ketone although no carbonyl derivatives have been obtained.

Experiments designed to avoid elimination of halogen during periodate oxidation

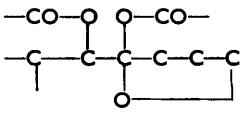
and to obtain more conclusive evidence on the fate of the carbonyl function generated are being actively pursued.

Table 4 summarises the action of periodic acid on the second reduction product of β-bromopicrotoxinin, $C_{15}H_{21}O_6Br$.

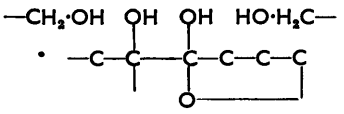
TABLE 4. *Periodic acid oxidation of reduced β-bromopicrotoxinin,* $C_{15}H_{21}O_6Br$.

Time	30 min.	2 hr.	24 hr.	45 hr.
Reagent reacted (atoms of O/mol.)	0·65	0·65	0·95	1·15
Acid formed (equiv./mol.)	0·5	0·5	0·8	—

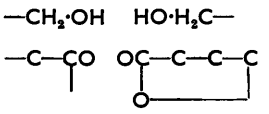
In previous papers of this series, especially Part IV,[1] attention has been drawn to the weakness of the evidence that the two titratable centres of acidity in picrotoxinin are the two lactone groups present. There is now increasing evidence that one may be an acidic hydroxyl group. Thus, (*a*) both bromopicrotoxinin and hydroxypicrotoxinin are monobasic only, the loss in each case of a hydroxyl group in the change from picrotoxinin being accompanied by loss of one unit of acidity, (*b*) the change of α- to β-picrotoxinic acid



(XI)

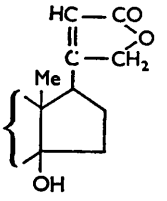


(XII)

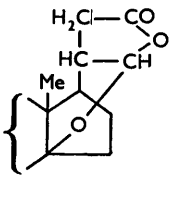


(XIII)

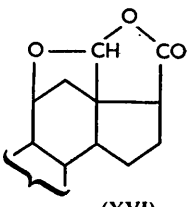
appears to sequester the hydroxyl group responsible for the " reducing " action of the former and this loss is accompanied by loss of one unit of acidity,[21] (*c*) reduced picrotoxinin, which lacks carbonyl absorption and cannot therefore be lactonic, still titrates as a monobasic substance, and (*d*) both picrotoxinin and reduced picrotoxinin yield methyl ethers



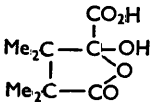
(XIV)



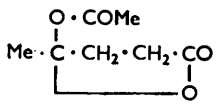
(XV)



(XVI)



(XVII)



(XVIII)

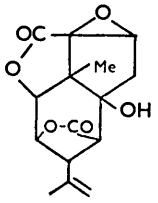
with diazomethane. If this view is correct it implies that one of the lactone groups of picrotoxinin and closely related compounds such as bromopicrotoxinin is stable to dilute aqueous alkali. This is readily intelligible if one of the lactones is of the hemiacetal type postulated above. Such a system is found in certain products derived from the cardiac glycosides; digitoxigenin and strophanthidin (XIV) give the " *iso* "-isomers (XV) under alkaline conditions, and the methods by which these compounds are prepared suggest that they are insoluble in dilute aqueous alkali, *i.e.*, that the hemiacetal-lactone group is stable under these conditions.[22] The grouping (XVI) also occurs in certain degradation products of the aldosteroids, and these likewise are not alkali-soluble.[23,24] Woodward and Eastman[25]

[21] Horrmann, *Ber.*, 1913, **46**, 2793.
[22] Ref. 17, p. 519.
[23] Simpson, Tait, Wettstein, Neher, von Euw, Schindler, and Reichstein, *Experientia*, 1954, **10**, 132.
[24] *Idem, Helv. Chim. Acta*, 1954, **37**, 1200.
[25] Woodward and Eastman, *J. Amer. Chem. Soc.*, 1950, **72**, 399.

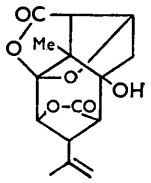
have drawn attention to the behaviour of the compound (XVII), which titrates as a monobasic acid.[26]

Picrotoxinin and related dilactonic compounds, examined in Nujol mull, all possess one lactone band in the infrared region at a higher wave number than that usually associated with saturated γ-lactones, *viz.*, 1780—1760 cm.$^{-1}$ (solution spectra):[27] thus for picrotoxinin, 1764; dihydropicrotoxinin, 1794; picrotoxinin epoxide, 1799; α-bromopicrotoxinin, 1784; β-bromopicrotoxinin, 1792; picrotin, 1803; *neo*picrotoxinin 1798; bromo*neo*picrotoxinin, 1797 cm.$^{-1}$. This frequency shift is observed in the simple hemiacetal-lactone γ-acetoxy-γ-valerolactone (XVIII), which absorbs[28] at 1797 cm.$^{-1}$; compounds in the series (XVI) absorb at the extreme of the normal range (1779 cm.$^{-1}$).

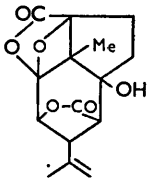
Further, if an ether link is present, the lithium aluminium hydride reductions show that it cannot be an ethylene oxide, as postulated by Conroy,[29] since this would suffer fission under such conditions. This view is supported by a comparison of the infrared spectra of picrotoxinin and its epoxide—that of the latter contains bands at 1234, 915, and 781 cm.$^{-1}$ attributable to the ethylene oxide ring but absent in the picrotoxinin spectrum (cf. Gunthard *et al.*[30]). It is also unlikely that any other simple ether linkage is present, since no such linkage is observed in any of the degradation products of known structure. On the other hand the hemiacetal link is not open to this criticism since it would be unlikely to survive vigorous degradation and it also provides a simple explanation of the very different effects of acid and alkali upon picrotoxinin.



(XIX)



(XX)



(XXI)

Although decisive degradative evidence is still lacking and the observed molecular rearrangements remain obscure, it is now possible to modify the Conroy formula (XIX) towards a more acceptable solution. This may be done (the sixth oxygen atom being assumed to be present in an ether linkage) by replacing the ethylene oxide ring by a hemiacetal-lactone system, as in (XX) or (XXI). Neither of these formulae is however completely satisfactory and additional work is required.

Experimental

Hydroxypicrotoxinin Formate (II).—17·1N-Hydrogen peroxide (2·0 ml.) was added to picrotoxinin (3·8 g.) in 98% formic acid (20 ml.), and the mixture allowed to evaporate at 35° overnight. The residue was crystallised from aqueous dioxan, to yield *hydroxypicrotoxinin formate* (2·28 g.). Recrystallisation gave the pure ester as needles, m. p. 237—238° (Found: C, 57·1; H, 4·7. $C_{16}H_{16}O_8$ requires C, 57·2; H, 4·8%). More crude ester (1·2 g.), m. p. 215—217°, was recovered from the mother-liquors.

Hydroxypicrotoxinin (III; R = H).—Hot 2·5% formic acid (20 ml.) was added to hydroxypicrotoxinin formate (0·5 g.) in hot dioxan (5 ml.), and the mixture refluxed for 1 hr. and then concentrated. The *hydroxypicrotoxinin* (0·35 g.), which gradually separated, was recrystallised from aqueous acetone, to give needles (grouped in plates resembling hoar frost), m. p. 249—250° (decomp.), $[\alpha]_D^{16}$ −90° (in acetone) (Found: C, 58·9; H, 5·65. $C_{15}H_{16}O_7$ requires C, 58·5; H, 5·25%). The *acetate* (III; R = Ac), prepared with acetic anhydride and anhydrous ferric chloride, crystallised from aqueous acetone in plates, m. p. 236° (Found: C, 58·1; H, 5·2; Ac, 13·5. $C_{17}H_{18}O_8$ requires C, 58·3; H, 5·2; Ac, 12·3%).

Dihydroxypicrotoxinin (VII).—(*a*) Perbenzoic acid (0·55 g., 1·05 mol.) in acetone (10 ml.) was

[26] Rothstein and Shoppee, *J.*, 1927, 531.
[27] Ref. 20, p. 153.
[28] Rasmussen and Brattain, *J. Amer. Chem. Soc.*, 1949, **71**, 1073.
[29] Conroy, *ibid.*, 1951, **73**, 1889.
[30] Gunthard, Heusser, and Furst, *Helv. Chim. Acta*, 1953, **36**, 1900.

added to picrotoxinin (1·1 g.) in acetone (40 ml.), and the solution kept at room temperature for 36 hr. and then allowed to evaporate. The solid residue, in chloroform, was diluted with ether, and the precipitate (0·75 g.) was crystallised several times from aqueous acetone, to give material, m. p. *ca.* 185° after much previous softening, which consisted largely of the epoxide (Found: C, 57·4; H, 5·8. Calc. for $C_{15}H_{16}O_7$: C, 58·5; H, 5·25%). It reacted with small quantities of lead tetra-acetate and of periodic acid. The mother-liquors from the crystallisation of the epoxide were concentrated to give a mixture of the epoxide and prisms of *dihydroxypicrotoxinin*. The former washed out with ether–acetone (1 : 1), and the latter, crystallised from aqueous acetone, had m. p. 262° (decomp.), $[\alpha]_D^{17}$ −65° (in acetone) (Found: C, 55·1; H, 5·45. $C_{15}H_{18}O_8$ requires C, 55·2; H, 5·55%). The compound consumed 1·01 mol. of periodic acid in aqueous methanol (1 : 1) during 1 hr. and 0·41 and 1·00 mol. of lead tetra-acetate in glacial acetic acid during 1 and 24 hr. respectively. The *triacetate*, prepared with acetic anhydride and anhydrous ferric chloride, crystallised from aqueous acetone as plates, m. p. 240° (Found: C, 56·1; H, 5·6; Ac, 28·1. $C_{21}H_{24}O_{11}$ requires C, 55·8; H, 5·35; 3Ac, 28·6%). (*b*) Perbenzoic acid (1·0 g.) in chloroform (16 ml.) was added to picrotoxinin (2·0 g.) in acetone (5 ml.), and the solution was allowed to evaporate overnight at 35°. The solid residue was dissolved in dioxan (10 ml.) and refluxed with 5% formic acid (40 ml.) for 1 hr. The resulting solution was washed with ether (3 × 20 ml.) and concentrated, to yield the dihydroxy-compound (1·6 g), identical with that described above.

α-Picrotoxinone (VIII).—Dihydroxypicrotoxinin (1·25 g.) was dissolved in hot water (20 ml.), cooled to about 50°, and treated with periodic acid (0·86 g., 1 mol.) in water (5 ml.). The mixture was kept at room temperature for 15 min. and then at 0° for 15 min. The crystals which separated (0·9 g.) were recrystallised from aqueous acetone to give α-picrotoxinone as needles, m. p. (variable according to conditions of heating) *ca.* 210—220°, $[\alpha]_D^{17}$ −10·3° (in EtOH) (Found: C, 56·8; H, 5·35. Calc. for $C_{14}H_{14}O_7$: C, 57·2; H, 4·8%). The ketone showed no depression of the m. p. when mixed with authentic α-picrotoxinone. The ketone was further characterised and identified by conversion into β-picrotoxinone (m. p. and mixed m. p. 252°) and picrotoxonic acid [m. p. and mixed m. p. 256° (decomp.)]. It gave a positive iodoform reaction.

The original filtrate from the α-picrotoxinone was neutralised with barium hydroxide, filtered, and distilled. Formaldehyde (0·71 mol.), estimated and identified as the dimedone derivative (m. p. and mixed m. p. 190°), was evolved.

Hydroxypicrotoxinic Acids.—(*a*) Sufficient dilute sodium hydroxide solution and water were added to hydroxypicrotoxinin (0·38 g.) in acetone (10 ml.) to give a homogeneous alkaline solution. The solution was set aside for 5 min., then made just acid (litmus) with hydrochloric acid and set aside for several days. The material which separated was taken up in the minimum quantity of dilute sodium hydroxide solution and acidified as above. The crystals which separated (0·30 g.) were recrystallised from water, to give the pure *acid*, m. p. 238—239° (decomp.), $[\alpha]_D^{18}$ −42° (in EtOH) (Found: C, 55·1; H, 5·9. $C_{15}H_{18}O_8$ requires C, 55·2; H, 5·55%). The acid did not react with lead tetra-acetate in acetic acid during 1 hr. (*b*) Sufficient dilute sodium hydroxide solution and water were added to hydroxypicrotoxinin formate (0·2 g.) in dioxan (5 ml.) to give a homogeneous alkaline solution. After 1 hr. the solution was made acid to Congo-red with hydrochloric acid, diluted with water (10 ml.), and concentrated to a thin syrup which deposited needles of hydroxypicrotoxinic acid (38 mg.) identical with the acid described above. (*c*) α-Picrotoxinic acid (0·7 g.) in acetone (5 ml.) was treated with perbenzoic acid (0·294 g.) in chloroform (7 ml.), and the mixture allowed to evaporate overnight. The crude solid was washed with ether and refluxed with dioxan (5 ml.) and 5% formic acid (20 ml.) for 2 hr. The solution was extracted several times with ether, concentrated, and kept for several days. The few crystals (*ca.* 20 mg.) which separated were identified by m. p. and mixed m. p. with the acid described above. (*d*) 17N-Hydrogen peroxide (0·4 ml.) was added to α-picrotoxinic acid (0·75 g.) in 95% formic acid (5 ml.), and the solution, after 5 hr. at 35°, was diluted with water (5 ml.) and left overnight. The solid formed (0·75 g.) crystallised from water, to give the isomeric *hydroxypicrotoxinic* acid as needles, m. p. 227° (decomp.), $[\alpha]_D^{18}$ −47·5° (in EtOH) (Found: C, 55·9; H, 6·2%). The acid did not react with lead tetra-acetate in acetic acid during 24 hr.

Acetylhydroxyneopicrotoxinin.—(*a*) Solutions of acetyl*neo*picrotoxinin (0·4 g.) in acetone (5 ml.) and of perbenzoic acid (0·18 g.) in chloroform (3·6 ml.) were mixed and kept at 25° for 5 hr. The solid residue, dissolved in acetone (5 ml.), was mixed with water (5 ml.) and 98% formic acid (1 ml.) and left at 25° for 10 hr. The residue was washed with ether (10 ml.), and the solid (0·346 g.) crystallised from aqueous acetone to give the *hydroxy-compound* as needles, m. p. 251—252° (Found: C, 58·8; H, 5·55; Ac, 11·9, 12·6. $C_{17}H_{18}O_8$ requires C, 58·3; H, 5·2;

Ac, 12·3%). It was recovered from treatment with periodic acid, formic acid, and sodium methoxide in methanol, and did not react with lead tetra-acetate in acetic acid during 1 hr. It readily reduced sensitised ammoniacal silver nitrate. (*b*) Acetyl*neo*picrotoxinin (0·25 g.), 17N-hydrogen peroxide (0·15 ml.), and 98% formic acid (3 ml.) were set aside overnight. The residue was repeatedly crystallised from aqueous acetone to give needles, m. p. 244°, showing no depression when mixed with the above hydroxy-compound (Found: C, 59·1; H, 5·55; Ac, 15·5%).

Reduction of Methyl β-Bromopicrotoxinate.—Methyl β-bromopicrotoxinate (21·8 g.) in dry dioxan (100 ml.) was added dropwise with stirring to a suspension of lithium aluminium hydride (11 g.) in dry ether (200 ml.), and the mixture refluxed for 30 min. Ethyl acetate (20 ml.) was added with cooling, followed by 2N-sulphuric acid (400 ml.). Solid sodium hydrogen carbonate was added until the mixture was just alkaline to litmus, and the whole was then evaporated to dryness under reduced pressure. The residue was refluxed with successive portions of absolute ethanol (5 × 200 ml.), and the combined extracts were evaporated under reduced pressure. The solid residue separated from water as crystals, m. p. 176° (12·5 g., 58%), raised by further crystallisation from water to 178°, $[\alpha]_D^{18}$ −90° (in EtOH) (Found, for hydrated material: C, 45·8; H, 5·75; Br, 20·4. $C_{15}H_{21}O_6Br,H_2O$ requires C, 45·6; H, 5·85; Br, 20·3. Found, for anhydrous material: C, 47·9, 47·8; H, 5·65, 5·7. $C_{15}H_{21}O_6Br$ requires C, 47·8; H, 5·6%). The *alcohol* did not reduce ammoniacal silver nitrate solution. It reacted with 0·48 and 0·98 mol. of lead tetra-acetate in acetic acid during 1 and 24 hr. respectively. Diazomethane is without effect on the compound, and conductometric titration failed to reveal any potential acidity. The same compound was obtained by reducing β-bromopicrotoxinic acid.

Reduction of β-Bromopicrotoxinin.—β-Bromopicrotoxinin (20 g.) was reduced with lithium aluminium hydride (11 g.) as described above. The crude solid (generally 5·2—6·2 g., m. p. *ca.* 210°) was crystallised from ethanol or water, to give the pure *alcohol*, m. p. 219° (decomp.), $[\alpha]_D^{19}$ −112° (in EtOH) (Found: C, 48·0, 47·95; H, 6·4, 6·25; Br, 21·0, 22·95. $C_{15}H_{23}O_6Br$ requires C, 47·5; H, 6·1; Br, 21·2%). The alcohol gave an amorphous p-*nitrobenzoate* (Found: C, 52·5, 52·6; H, 3·4, 3·9; N, 5·3. $C_{36}H_{32}O_{15}N_3Br$ requires C, 52·3; H, 3·9; N, 5·1%), absorption bands (3μ region) at 3485 and 3455 cm.$^{-1}$ (no CO absorption).

A portion of the mother-liquor from which the crude alcohol had separated was dissolved in dilute aqueous sodium hydroxide and continuously extracted with ether. The ether extract yielded a neutral syrup which deposited a little of the crystalline alcohol on long storage. The alkaline solution was then acidified and extracted with ether. The extract yielded a syrup which slowly deposited crystals, further crystallised from ethanol to yield a second *alcohol*, m. p. (for two specimens) 203°, 205° (decomp.) (Found: C, 47·45; H, 5·5. $C_{15}H_{21}O_6Br$ requires C, 47·75; H, 5·6%). The compound lacked absorption in the carbonyl region of the infrared spectrum. It reacted with 0·55 mol. of lead tetra-acetate in acetic acid during 1 or 24 hr.

Reduction of Picrotoxinin.—Picrotoxinin was reduced with lithium aluminium hydride as described above, and the crude syrup from several preparations chromatographed on alumina. One fraction, which reacted with 0·49 mol. of lead tetra-acetate in acetic acid during 1 hr., deposited a small quantity of a *compound* which formed large plates (from methanol), m. p. 212° (Found: C, 60·85, 61·1, 60·85, 60·65; H, 7·25, 7·25, 6·9, 6·9. $C_{15}H_{20}O_6$ requires C, 60·8; H, 6·8%).

Methylation of Reduced Picrotoxinin.—(*a*) Ethereal diazomethane was added to the alcohol (50 mg.), dissolved in methanol (10 ml.), to yield the *methyl ether* which crystallised from methanol in needles (16 mg.), m. p. 170° (Found: C, 62·4; H, 7·09. $C_{16}H_{22}O_6$ requires C, 61·9; H, 7·1%). (*b*) The alcohol (57 mg.) was dissolved in the minimum quantity of 40% aqueous potassium hydroxide, diluted with a little water, warmed slightly, and then treated with methyl sulphate (4 drops), the mixture remaining alkaline. The solution was warmed for a few minutes, acidified with sulphuric acid, and extracted five times with ethyl acetate. The extract was evaporated, to yield a solid which crystallised from ethanol (yield, 10 mg.) and then aqueous ethanol and had m. p. 176° [mixed m. p. with the product from (*a*), 171°].

Hydrogenation of Reduced Picrotoxinin.—The alcohol was hydrogenated in the presence of platinum to give the *derivative* (Found: C, 60·3; H, 7·68. $C_{15}H_{22}O_6$ requires C, 60·4; H, 7·4%).

Attempted Debromination of Reduced Bromopicrotoxinin.—The reduced bromopicrotoxinin was treated with zinc dust and ammonium chloride in the usual way, to give a syrup from which, by conventional methods, small quantities of a non-reducing substance, m. p. 226°, were obtained. By working up mother-liquors a second compound was obtained as needles (from methanol), m. p. 153° (Found: C, 59·9; H, 7·05. Calc. for $C_{15}H_{22}O_6$: C, 60·4; H, 7·4.

Calc. for $C_{15}H_{24}O_6$: C, 60·0; H, 8·05%), which did not react with lead tetra-acetate in acetic acid.

Reaction of Reduced β-Bromopicrotoxinic Acid with Periodic Acid.—A solution of periodic acid (7·0 g.) in water (35 ml.) was shaken with the reduction product (7·6 g.) in water (75 ml.) at 50°. The resulting semisolid mass, after 30 min. at 0°, was filtered off and washed with water. The *ketone* (7·25 g.) crystallised from water in needles, m. p. 200—201°, $[\alpha]_D^{18}$ −46·5° (in acetone) (Found: C, 48·85, 48·8; H, 5·45, 5·0; O, 22·9; Br, 22·8. $C_{14}H_{17}O_5Br$ requires C, 48·7; H, 4·9; O, 23·2; Br, 23·2%). It does not reduce ammoniacal silver nitrate, is unaffected by diazomethane, and gives a 2 : 4-*dinitrophenylhydrazone*, sparingly soluble in ethanol from which it crystallises in yellow needles, m. p. 282° (decomp.) (Found: C, 46·1; H, 4·0; N, 11·1. $C_{20}H_{21}O_8N_4Br$ requires C, 45·7; H, 4·0; N, 10·7%). The ultraviolet spectrum of the ketone shows a maximum at 302—305 mμ (ε 31).

The filtrate from the crude ketone was neutralised with barium hydroxide solution, filtered, and distilled. The distillate yielded the dimedone derivative of formaldehyde, m. p. and mixed m. p. 187° (0·09 mol.).

Reduction of the Ketone.—The ketone (0·75 g.) was reduced with lithium aluminium hydride to a product which crystallised in needles (0·46 g.) from water. Recrystallised, the *alcohol* had m. p. 174° and showed a marked depression when mixed with the reduction product, m. p. 178°, of β-bromopicrotoxinic acid itself (Found: C, 49·0; H, 5·9; Br, 21·3, 21·6. $C_{14}H_{19}O_5Br$ requires C, 48·4; H, 5·5; Br, 23·05%).

Reaction of Reduced β-Bromopicrotoxinin with Periodic Acid.—The alcohol $C_{15}H_{23}O_6Br$ (1·0 g.) was shaken with a solution of periodic acid (1·4 g.) in water (40 ml.) until dissolution was complete and then left overnight. During the reaction bromine was liberated. The solution was made just alkaline with barium hydroxide, filtered, acidified with a few drops of dilute sulphuric acid, filtered again, and then concentrated to half-volume under slightly reduced pressure. The concentrate was continuously extracted with ether to give, after removal of the ether, a glass (0·45 g.) which partially crystallised. The extraction was continued with ethyl acetate to give two fractions of which the first was solid (0·24 g.) and the second partly crystalline (0·26 g.). The solid products were combined and crystallised from water or ethanol, to give the *lactone*, m. p. 255° (decomp.), $[\alpha]_D^{18}$ −85° (in EtOH) (Found: C, 57·55, 57·05, 57·05; H, 6·85, 7·05, 6·85. $C_{15}H_{22}O_7$ requires C, 57·3; H, 7·05%). The lactone did not reduce ammoniacal silver nitrate or react with diazomethane.

A small quantity of a second product was obtained in one experiment as flat needles or rectangular plates (from ethanol), m. p. 227°, $[\alpha]_D^{20}$ −116° ± 13° (in EtOH) (Found: C, 60·45; H, 8·25. $C_{15}H_{24}O_6$ requires C, 60·0; H, 8·05%). It showed a mixed m. p. depression with the product, m. p. 226°, obtained in the debromination of reduced β-bromopicrotoxinin.

Acetylation of Picrotin.—(a) *With acetic anhydride and sulphuric acid.* Picrotin (2 g.), acetic anhydride (10 ml.), and concentrated sulphuric acid (2 drops) were set aside for 2 days and the resulting white precipitate (0·43 g.) was filtered off and recrystallised from much methanol; it then had m. p. >300°. The filtrate was decomposed with water, and the resulting precipitate (0·81 g.) was collected and fractionally crystallised from methanol to yield acetyl-*neo*picrotoxinin, m. p. and mixed m. p. 190° (Found: C, 60·7; H, 4·9; Ac, 12·6. Calc. for $C_{17}H_{18}O_7$: C, 61·1; H, 5·4; Ac, 12·9%), and a higher-melting, more soluble *isomer*, m. p. 245° sharp depression when mixed with monoacetylpicrotin of similar m. p. (Found: C, 60·6; H, 5·45; Ac, 12·3%). The latter compound was unsaturated towards alkaline permanganate.

(b) *With acetic anhydride and ferric chloride.* A small quantity of anhydrous ferric chloride was added to picrotin in excess of acetic anhydride. After 5 days the product was diluted with water, to yield the diacetate, which, crystallised from ethanol, had m. p. 200° (Found: C, 57·9; H, 5·6; Ac, 21·9. Calc. for $C_{19}H_{22}O_9$: C, 57·9; H, 5·6; 2Ac, 21·8%). If the reaction mixture is heated on the water-bath the main product is anhydropicrotin, m. p. and mixed m. p. 328° (Found: C, 62·0; H, 5·3. Calc. for $C_{15}H_{16}O_6$: C, 61·6; H, 5·5%).

Reaction between α-Picrotoxinone and Diazomethane (with GHULAM HASSAN and G. R. SLEEMAN).—Ethereal diazomethane (slight excess) was added to α-picrotoxinone (0·4 g.) in methanol. After 2 hr. the solvent was removed under reduced pressure and the residue crystallised from methanol. The *ether* (0·39 g.) had m. p. *ca.* 202° (gas evolution; bath pre-heated to 190°) (Found: C, 54·8, 54·8; H, 5·2, 5·3; N, 8·1, 7·8; OMe, 9·6. $C_{16}H_{18}O_7N_2$ requires C, 54·85; H, 5·2; N, 8·0; OMe, 8·9%).

Reaction between β-Picrotoxinone and Diazomethane.—Under conditions similar to those described above a product was obtained, m. p. *ca.* 198° (gas evolution), mixed m. p. with the above substance, *ca.* 195° (Found: N, 8·1%).

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