607. *Picrotoxin. Part VII.* The Chemistry of Anhydropicrotin.*

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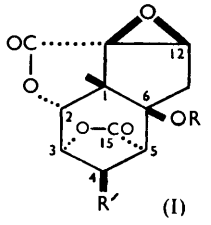
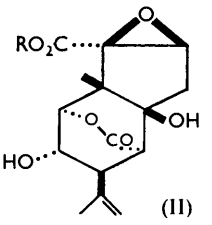
An investigation on the formation and properties of anhydropicrotin and the derived anhydropicrotic acid has led us to propose structures (IV; R = H) and (VI; R = R′ = R″ = H) respectively for these compounds. The identity of anhydropicrotic and β-picrotoxinic acid has been established. Anhydropicrotin has been converted into *neo*picrotoxinin acetate and benzoate, and a re-investigation of the ozonolysis of this acetate has shown it to contain an *iso*propylidene group. Evidence is presented in favour of structures (X; R = :CMe_2, R′ = H) and (XII) for *neo*picrotoxinin and β-dihydropicrotoxinin respectively.

Mechanisms are suggested for the ready conversion of *neo*picrotoxinin into the aromatic compound picrotonol (XVIII), and for the formation of picrotoxinin and *neo*picrotoxinin acetates, and of anhydropicrotin by treatment of picrotin with acetic anhydride containing sulphuric acid.

The molecular compound picrotoxin, $C_{30}H_{34}O_{13}$, is readily separable into its components picrotoxinin, $C_{15}H_{16}O_6$, and picrotin, $C_{15}H_{18}O_7$.[1] Despite the recent structural elucidation of picrotoxinin (I; R = H, R′ = ·CMe:CH_2),[2,3] α-picrotoxinic acid (II; R = H),[2] their related bromo-derivatives,[4] and picrotoxic acid (III),[3,5] there are still many transformation products of picrotoxinin and picrotin for which the structural evidence is vague. In continuation of our studies of these products we have now investigated the reactions of anhydropicrotin and *neo*picrotoxinin and the formation of these compounds and their derivatives from picrotoxinin and picrotin.



(I)



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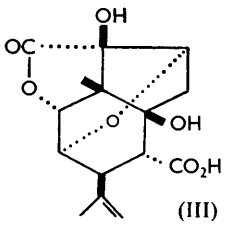


(III)

In an attempt to convert picrotin, $C_{15}H_{18}O_7$, into picrotoxinin (I; R = H, R′ = CMe:CH_2) Horrmann [6] dehydrated the former compound with phosphorus pentachloride in chloroform and obtained an isomeride of picrotoxinin which he named anhydropicrotin. We have now shown that picrotoxinin can also be converted into anhydropicrotin with hydrogen chloride in acetic acid at room temperature or with boiling 95% formic acid.

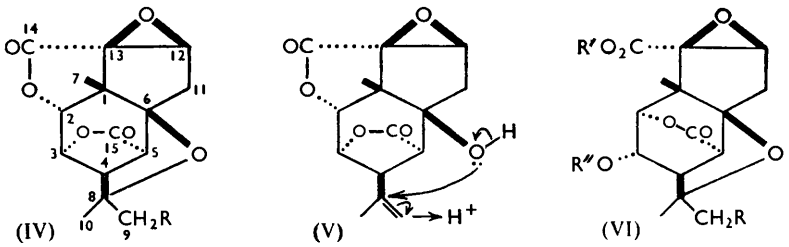
Anhydropicrotin does not react with ozone, bromine, or hydrogen in the presence of catalysts, and the infrared spectrum of this compound, although similar to that of picrotoxinin in the carbonyl stretching region, shows a total absence of double bond and hydroxyl absorptions. It therefore seems likely that in the conversion of picrotoxinin into anhydropicrotin the tertiary hydroxyl group and the *iso*propenyl double bond of the former compound have been transformed. This hypothesis is supported by the fact that picrotoxinin derivatives which lack either of these functional groups do not undergo

* Part VI, *J.*, 1957, 4945.

[1] Horrmann and Prillwitz, *Arch. Pharm.*, 1920, **258**, 200.
[2] Conroy, *J. Amer. Chem. Soc.*, 1951, **73**, 1889; 1952, **74**, 491.
[3] *Idem, ibid.*, 1957, **79**, 5551.
[4] *Idem, ibid.*, p. 1726.
[5] Burkhill, Holker, Robertson, and Taylor, *J.*, 1957, 4945.
[6] Horrmann, *Ber.*, 1910, **43**, 1903.

parallel reactions; thus, α-dihydropicrotoxinin (I; R = H, R′ = ·CHMe$_2$) and picrotoxinin acetate (I; R = Ac, R′ = ·CMe:CH$_2$) were recovered unchanged after attempted isomerisation. Conroy [4] has shown that bonding between the *iso*propenyl and the tertiary hydroxyl group of picrotoxinin does occur when the compound is brominated, to give bromopicrotoxinin (IV; R = Br). It therefore seemed likely that anhydropicrotin has a similar structure (IV; R = H) and is formed from picrotoxinin by the concerted mechanism shown in (V).

Horrmann [6] demonstrated that with dilute aqueous sodium hydroxide anhydropicrotin gave the monocarboxylic anhydropicrotic acid, $C_{15}H_{18}O_7$, characterised as its methyl ester. We have repeated this preparation and find that anhydropicrotic acid is identical with β-picrotoxinic acid isolated by Horrmann [7] by isomerisation of α-picrotoxinic acid (II; R = H) with 2N-sulphuric acid. Comparison of the methyl esters derived from the two acid samples confirms the identity. Thus, on the basis of structures (IV; R = H) for anhydropicrotin and (II; R = H) for α-picrotoxinic acid, β-picrotoxinic acid has structure (VI; R = R′ = R″ = H) and its ester, which can be conveniently prepared directly from anhydropicrotin by methanolysis, has structure (VI; R = R″ = H, R′ = Me). In agreement with this, methyl β-picrotoxinate showed bands at 3490 (OH), 1736 (δ-lactone), and 1720 cm.$^{-1}$ (CO_2Me) in its infrared spectrum and readily gave a monoacetate (VI; R = H, R′ = Me, R″ = Ac) which showed no hydroxyl absorption. It should be noted that the isomerisation of α-picrotoxinic to β-picrotoxinic acid would be expected to be mechanistically similar to that of picrotoxinin to anhydropicrotin.

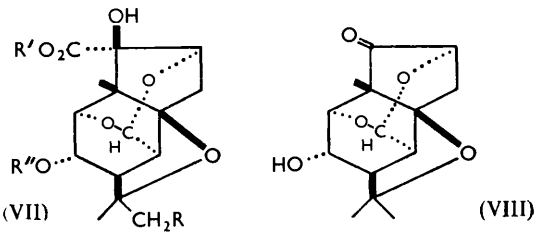


Reduction of methyl β-picrotoxinate with sodium borohydride gave a dihydro-derivative, methyl dihydro-β-picrotoxinate, showing infrared bands at 3520, 3490 (OH), and 1718 cm.$^{-1}$ (CO_2Me), but no absorption which could be attributed to a δ-lactone system. This compound formed a monoacetate having hydroxyl absorption at 3455 cm.$^{-1}$ and it is therefore apparent that methyl dihydro-β-picrotoxinate contains at least two hydroxyl groups, of which one can be readily acetylated. Treatment of methyl dihydro-β-picrotoxinate with 2N-sodium hydroxide generated dihydro-β-picrotoxinic acid which was reconverted into its ester on treatment with diazomethane. Dihydro-β-picrotoxinic acid was shown to be an α-hydroxy-acid by oxidation with lead dioxide in acetic acid to give carbon dioxide and a non-acidic ketone, $C_{14}H_{18}O_5$, characterised as its 2 : 4-dinitrophenylhydrazone. The infrared spectrum of this ketone showed bands at 3427 (OH) and 1767 cm.$^{-1}$ (attributed to a keto-group in a five-membered ring). Conroy [4] has shown that reduction of β-bromopicrotoxinic acid (VI; R = Br, R′ = R″ = H) with sodium borohydride gave a dihydro-derivative which was shown to be an α-hydroxy-acid by oxidation with lead dioxide in acetic acid to a non-acidic ketone, $C_{14}H_{19}O_5Br$, and carbon dioxide. These reactions have been interpreted by Conroy on the basis of structure (VII; R = Br, R′ = R″ = H) for β-bromo-dihydropicrotoxinic acid, which is considered to be formed from β-bromopicrotoxinic acid by attack of the borohydride anion on the carbon atom of the lactonic 14-carbonyl group, with simultaneous rearward displacement of the epoxide ring by the accumulating negative charge on the carbonyl oxygen atom. The striking similarities between the formation and properties of dihydro-β-picrotoxinic and

[7] Horrmann, *Ber.*, 1913, **46**, 2793.

dihydro-β-bromopicrotoxinic acid indicate that these reduction products have similar structures; consequently methyl dihydro-β-picrotoxinate, its acetate, dihydro-β-picrotoxinic acid, and the ketone derived by lead dioxide oxidation are formulated as (VII; R = R″ = H, R′ = Me), (VII; R = H, R′ = Me, R″ = Ac), (VII; R = R′ = R″ = H), and (VIII) respectively. This work also provides further support for structure (VI; R = R″ = H, R′ = Me) for methyl β-picrotoxinate.

It was reported by Horrmann [6] that treatment of anhydropicrotin with boiling benzoyl chloride gave a monobenzoate, $C_{22}H_{20}O_7$, whereas with boiling acetic anhydride and acetyl chloride a mixture of a monoacetate and a diacetate was formed. We have repeated the preparation of the monobenzoate by Horrmann's method but have been unable to effect the acetylation by his method, unchanged material being recovered in

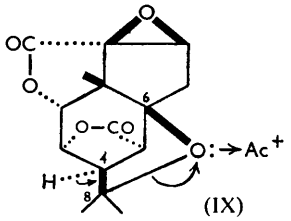


high yield. Treatment of anhydropicrotin with acetic anhydride and ferric chloride, however, gave a monoacetate, $C_{17}H_{18}O_7$, which had the same melting point as that reported by Horrmann for his diacetate. We have been unable to prepare a diacetate. Further investigation of our acetate showed it to be identical with *neo*picrotoxinin acetate which was first isolated by O'Donnell, Robertson, and Harland [8] from the mixture produced on treatment of a solution of picrotoxinin in acetic acid with hydrogen in the presence of a palladium catalyst and subsequent acetylation. *neo*Picrotoxinin and its acetate were further investigated by Slater *et al.*[9,10] who suggested that these compounds contain an *iso*propylidene group. This has now been confirmed by ozonolysis of *neo*picrotoxinin acetate in ethyl acetate solution to give a crystalline ozonide, m. p. 182—184° (decomp.), $C_{17}H_{18}O_{10}$, which was not decomposed by treatment with water and readily recrystallised from alcohol without decomposition. Treatment of the ozonide in ethyl acetate with hydrogen in the presence of a platinum catalyst brought about scission into acetone and a compound which analysed satisfactorily for the expected formula, $C_{14}H_{12}O_8$, after thorough drying. There seems to be little doubt that the ozonolysis product, m. p. 182—184° (decomp.), isolated by Slater [9] from *neo*picrotoxinin acetate and regarded by him as being a scission product, $C_{12}H_{12}O_7$, was the above stable ozonide, the analytical figures reported being compatible with this formulation.

The presence of an *iso*propylidene group in *neo*picrotoxinin acetate implies that its formation from anhydropicrotin (IV; R = H) involves electrophilic attack of the acetylium ion on the oxygen atom bridging positions 6 and 8, with subsequent loss of a proton from position 4, as in (IX), a reaction which is analogous to the opening of tetrahydrofurans with acetic anhydride in the presence of zinc chloride.[11] It thus appears that *neo*picrotoxinin acetate has structure (X; R = :CMe_2, R′ = Ac) and in its formation from anhydropicrotin the five-membered ether ring is opened in preference to the epoxide ring. This is in agreement with the observed remarkable stability of the epoxide ring in all picrotoxinin derivatives and has been attributed by Conroy [3] to the proximity of the 15-lactone bridge to the rear of the oxide ring, affording protection against rearward attack.

[8] O'Donnell, Robertson, and Harland, *J.*, 1939, 1261.
[9] Slater, *J.*, 1949, 806.
[10] Johns, Slater, Woods, Brasch, and Gee, *J.*, 1956, 4715.
[11] Elderfield, " Heterocyclic Compounds," Chapman and Hall, Ltd., London, 1950, Vol. I, p. 176.

On the basis of structure (X; R = :CMe_2, R′ = Ac) for *neo*picrotoxinin acetate, the derived ozonolysis product would be expected to have structure (X; R = O, R′ = Ac). However, this compound did not give a 2 : 4-dinitrophenylhydrazone and attempts to form an oxime produced an intractable water-soluble product which appeared to be a



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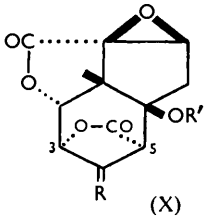

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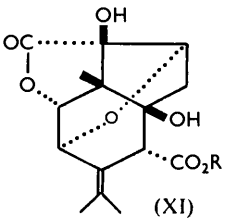


(XI)

hydroxamic acid since it gave a ruby-red ferric reaction. The infrared spectrum of the ozonolysis product, $C_{14}H_{12}O_8$, showed a band at 1701 cm.$^{-1}$, absent from the spectrum of *neo*picrotoxinin acetate and attributed therefore to an introduced ketonic carbonyl function. However, this frequency seems low for a ketone of type (X; R = O, R′ = Ac) in which the ketonic carbonyl group is present in a five-membered lactone ring. We hope to investigate further these unexpected properties and also the remarkable stability of the ozonide, but in the meantime, the evidence in favour of structure (X; R = :CMe_2, R′ = Ac) for *neo*picrotoxinin acetate, and hence, structure (X; R = :CMe_2, R′ = H) for *neo*picrotoxinin seems conclusive.

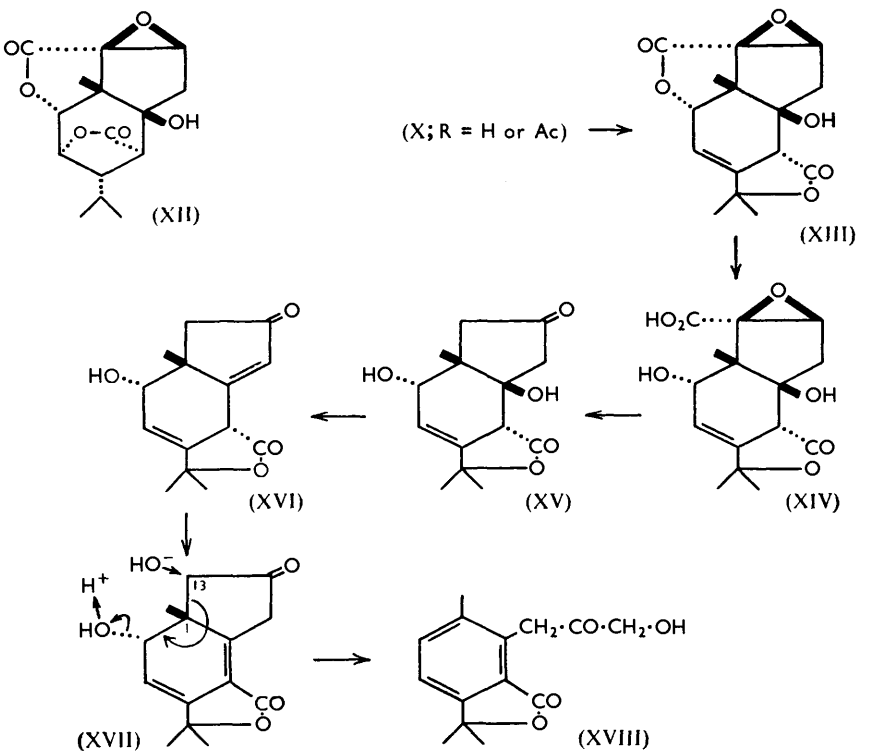
By analogy with *neo*picrotoxinin acetate, the benzoate derived from anhydropicrotin would be expected to have structure (X; R = Bz). This was supported by hydrolysis of both the acetate and the benzoate with dilute sodium hydroxide to the same crystalline acid, $C_{15}H_{18}O_7$, which gave a monomethyl ester, $C_{16}H_{20}O_7$, on treatment with diazomethane. This ester was in many respects similar to its isomer methyl picrotoxate: [5] it formed a monoacetate, decolorised bromine water without forming an insoluble monobromo-product, and was soluble in dilute sodium hydroxide solution, being reprecipitated on acidification. Its infrared spectrum showed bands at 1789 (γ-lactone) and 1738 cm.$^{-1}$ (CO_2Me). By analogy with methyl picrotoxate, the ester is named methyl *neo*picrotoxate and is tentatively formulated as (XI; R = Me). From conformational principles this type of formulation would be expected on the grounds that in both *neo*picrotoxinin acetate and picrotoxinin the oxygen atom at $C_{(3)}$ is similarly situated with respect to the epoxide ring, and therefore in the formation of both *neo*picrotoxic and picrotoxic acid the 3-hydroxyl group generated on opening of the lactone ring bridging positions 3 and 5 is in a suitable position for rearward attack at $C_{(12)}$ of the epoxide ring by an internal S_N2 mechanism.[5]

O'Donnell *et al.*[8] and subsequently Slater *et al.*[9,10] have shown that hydrogenation of picrotoxinin in the presence of a palladium–charcoal catalyst gives a mixture of two dihydro-derivatives, α- and β-dihydropicrotoxinin, together with the picrotoxinin isomer *neo*picrotoxinin (X; R = :CMe_2, R′ = H). Hydrogenation of picrotoxinin in the presence of a platinum catalyst gives α-dihydropicrotoxinin as the sole product. It seems clear that in the palladium-catalysed hydrogenation of picrotoxinin there are two competing reactions: (*a*) saturation of the *iso*propenyl system to give α-dihydropicrotoxinin (I; R = H, R′ = ·$CHMe_2$) and (*b*) isomerisation of the *iso*propenyl group to an *iso*propylidene group, leading to *neo*picrotoxinin; the latter reaction has analogies in the steroid field, *e.g.*, the isomerisation of cholest-7-enol to cholest-8(14)-enol in the presence of hydrogen and a palladium catalyst.[12] It has been shown [9] that β-dihydropicrotoxinin formed in the above palladium-catalysed hydrogenation is derived by slow saturation of the double bond in *neo*picrotoxinin. In the latter reaction it would be expected that addition of hydrogen to the double bond would occur from the least hindered side of the

[12] Wieland and Benend, *Annalen*, 1943, **554**, 1.

*neo*picrotoxinin molecule, *i.e.*, the face remote from the lactone ring bridging $C_{(3)}$ and $C_{(5)}$, thus leading to structure (XII) for β-dihydropicrotoxinin. Accordingly, this compound differs from the α-isomer only in the configuration of the *iso*propyl group at position 4. Models show that in picrotoxinin (I; R = H, R′ = ·CMe:CH_2) and α-dihydropicrotoxinin (I; R = H, R′ = ·$CHMe_2$) the tertiary hydroxyl group is sterically hindered by the *iso*propenyl and the *iso*propyl group respectively, whereas in *neo*picrotoxinin (X; R = :CMe_2, R′ = H) and β-dihydropicrotoxinin (XII) this hindrance is absent. It is thus clear why *neo*picrotoxinin and β-dihydropicrotoxinin can be readily acetylated [9] whereas picrotoxinin and α-dihydropicrotoxinin are acetylated only under forcing conditions.

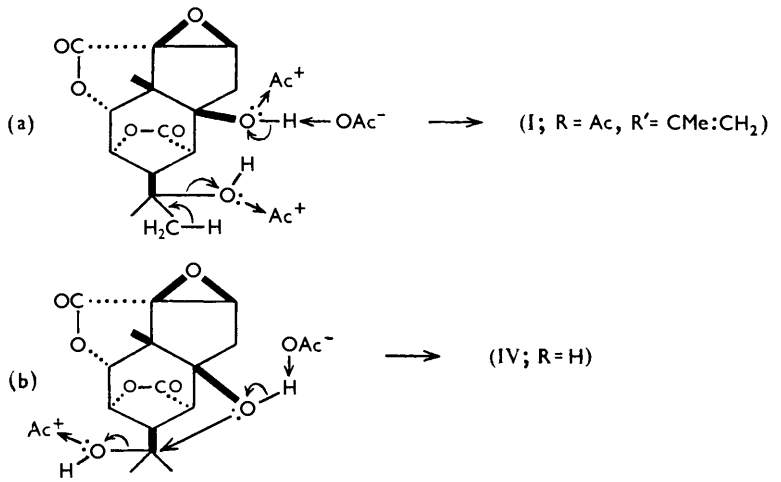
An interesting difference between the properties of picrotoxinin and its *neo*-isomer is the behaviour of these compounds with 2N-sulphuric acid. The former compound gives picrotoxic acid (III), a reaction we have already discussed,[5] whereas the latter or its acetate gives the aromatic compound picrotonol (XVIII) in high yield.[8] In this aromatisation it is considered that the driving force is probably allylic rearrangement of the 15-lactone in *neo*picrotoxinin (X; R = :CMe_2, R′ = H) to the intermediate (XIII) which is then converted into the diene (XVI) by way of the glycidic acid (XIV) and the β-hydroxy-ketone (XV). The resulting diene (XVI) or (XVII) is then aromatised by fission of the 1 : 13-bond in a carbonium-ion rearrangement, leading to picrotonol (XVIII).



Johns *et al.*[10] reported that picrotin reacts with acetic anhydride containing a trace of sulphuric acid to give *neo*picrotoxinin acetate, an unidentified monoacetate, $C_{17}H_{18}O_7$, and an insoluble compound, $C_{15}H_{16}O_6$. We find that the course of this heterogeneous reaction appears to be determined by the state of subdivision of the picrotin used. Thus, reaction of recrystallised picrotin with acetic anhydride and sulphuric acid gave a mixture from which anhydropicrotin (IV; R = H) and *neo*picrotoxinin acetate (X; R = :CMe_2, R′ = Ac) were isolated, whereas finely divided picrotin (precipitated from sodium

hydroxide with hydrochloric acid) gave a mixture from which anhydropicrotin and picrotoxinin acetate (I; R = Ac, R′ = ·CMe:CH_2) were isolated. It seems likely that the unidentified monoacetate and the " insoluble compound " of Johns *et al.* are identical with picrotoxinin acetate and anhydropicrotin respectively. Treatment of picrotin with boiling benzoyl chloride gave a mixture of *neo*picrotoxinin benzoate and anhydropicrotin.

The formation of both picrotoxinin and *neo*picrotoxinin derivatives directly from picrotin provides conclusive confirmatory evidence that the latter compound has structure (I; R = H, R′ = ·CMe_2·OH) in which the stereochemistry is identical with that of picrotoxin. It appears that picrotoxinin acetate and anhydropicrotin are formed from picrotin by competing dehydration mechanisms depicted in the annexed schemes (a) and (b) respectively, although it is not clear whether *neo*picrotoxinin acetate arises from anhydropicrotin produced in the reaction or by direct dehydration of picrotin with elimination of hydrogen from $C_{(4)}$. It should be noted that the formation of picrotoxinin acetate from picrotin appears to be the first recorded instance in which the latter compound has been converted directly into a picrotoxinin derivative containing an *iso*propenyl group.



Experimental

Ultraviolet absorption spectra were measured for 95% alcoholic solutions with a Unicam spectrophotometer and infrared spectra for mineral oil mulls with a Perkin-Elmer model 21 instrument. The light petroleum used had b. p. 60—80°.

Anhydropicrotin (IV; R = H).—(*a*) Prepared from picrotin with phosphorus pentachloride,[6] anhydropicrotin separated from acetic acid in rhombs, m. p. 322—324° (decomp.), $[\alpha]_D^{21}$ −99° (*c* 0·09 in AcOH), $\nu_{max.}$ 1783 and 1767 (shoulder) cm.$^{-1}$ (Found: C, 61·8; H, 5·4. Calc. for $C_{15}H_{16}O_6$: C, 61·6; H, 5·5%).

(*b*) A solution of picrotoxinin (1 g.) in 98% formic acid (9 ml.) was heated under reflux for 45 min. and poured into water (25 ml.). The precipitated solid was collected after 1 hr. and crystallised from acetic acid, to give anhydropicrotin in rhombs (0·4 g.), m. p. and mixed m. p. 323—325° (decomp.), $[\alpha]_D^{24}$ −100° (*c* 0·13 in AcOH). After removal of the above precipitate, the diluted reaction mixture was neutralised with sodium hydrogen carbonate and continuously extracted with ether, giving unchanged picrotoxinin, needles (0·4 g.), m. p. and mixed m. p. 204—206° (from water).

(*c*) Picrotoxinin (1 g.) in acetic acid (10 ml.) was saturated with hydrogen chloride at room temperature, then set aside overnight, and the crystals which separated were collected and recrystallised from acetic acid, to give anhydropicrotin in rhombs (0·7 g.), m. p. and mixed m. p. 324—326° (decomp.), $[\alpha]_D^{26}$ −101·5° (*c* 0·19 in AcOH). Evaporation of the acetic acid–hydrogen chloride filtrate gave unchanged picrotoxinin, needles (0·15 g.), m. p. and mixed m. p. 204—206° (from water).

Attempted isomerization of α-dihydropicrotoxinin and picrotoxinin acetate by hydrogen chloride in acetic acid as above gave unchanged starting materials in theoretical yield.

β-Picrotoxinic Acid (VI; R = R′ = R″ = H).—(*a*) Prepared from α-picrotoxinic acid with 2N-sulphuric acid,[7] β-picrotoxinic acid separated from water in needles, m. p. 233—234° (decomp.), $\nu_{max.}$ 1740 (δ-lactone) and 1718 cm.$^{-1}$ (CO_2H) (Found: C, 58·0; H, 6·1. Calc. for $C_{15}H_{18}O_7$: C, 58·1; H, 5·8%). Prepared with diazomethane the methyl ester separated from methanol in hexagonal plates, m. p. 226—227°, $[\alpha]_D^{19}$ −55° (*c* 1·17 in EtOH), $\nu_{max.}$ 3490 (OH), 1736 (δ-lactone), 1720 cm.$^{-1}$ (CO_2Me). Hydrolysis of the ester with aqueous 2N-sodium hydroxide regenerated β-picrotoxinic acid (m. p. and mixed m. p. 232—233°) in quantitative yield.

(*b*) Prepared from anhydropicrotin with 2N-sodium hydroxide according to Horrmann's procedure, β-picrotoxinic acid (anhydropicrotic acid) separated from acetone–benzene in needles, m. p. and mixed m. p. 231° (decomp.) [Horrmann [6] gives m. p. 221° (decomp.)]. The methyl ester formed plates, m. p. and mixed m. p. 228—230°, $[\alpha]_D^{18}$ −57° (*c* 0·89 in EtOH). The infrared spectra of this acid and its ester were identical with those of the corresponding acid and ester derived by method (*a*) above.

(*c*) To a suspension of finely powdered anhydropicrotin (1 g.) in methanol (50 ml.) was added a solution (0·7 ml.) of sodium methoxide in methanol (from 1 g. of sodium and 25 ml. of methanol), and the mixture was set aside until a homogeneous solution was formed (24 hr.). After acidification with acetic acid, the solution was evaporated, and the residue washed with water (80 ml.) and then crystallised from methanol, to give hexagonal plates (0·85 g.), m. p. 228—230°, identified as methyl β-picrotoxinate by mixed m. p. and infrared spectrum.

With acetic anhydride and pyridine methyl β-picrotoxinate gave a *monoacetate*, needles, m. p. 138—139° (from methanol) [Found: C, 59·0; H, 6·0; Ac, 11·3; OMe, 8·5. $C_{15}H_{16}O_5$(OAc)(OMe) requires C, 59·0; H, 6·0; Ac, 11·7; OMe, 8·5%].

Methyl Dihydro-β-picrotoxinate (VII; R = R″ = H, R′ = Me).—Methyl β-picrotoxinate (4 g.) in methanol (100 ml.) and water (50 ml.) was treated with potassium borohydride (2 g.) in water (15 ml.) at 45°. After 24 hr. at room temperature the mixture was acidified with 2N-hydrochloric acid, then concentrated (60 ml.), and the crude product (5—6 g.) isolated by continuous extraction with ether (4 days). To remove boron compounds, the product was dissolved in methanol (50 ml.) containing hydrogen chloride (2 g.), heated under reflux for 45 min., and neutralised with 2N-sodium hydrogen carbonate. *Methyl dihydro-β-picrotoxinate* was isolated by continuous extraction with ether (24 hr.), dried by azeotropic distillation with benzene, and crystallised from ethyl acetate–light petroleum, forming needles (1·9 g.), m. p. 233—234° (depressed to 191—194° on admixture with methyl β-picrotoxinate), $[\alpha]_D^{20}$ −67·8° (*c* 0·98 in EtOH), $\nu_{max.}$ 3520, 3490 (OH) and 1718 cm.$^{-1}$ (CO_2Me) (Found: C, 59·0; H, 7·0; OMe, 10·1. $C_{16}H_{22}O_7$ requires C, 58·9; H, 6·8; OMe, 9·5%). Methyl dihydro-β-picrotoxinate was immediately soluble in 2N-sodium hydroxide and was precipitated unchanged if immediately acidified. It did not react with periodic acid, ozone, or 2 : 4-dinitrophenylhydrazine hydrochloride. Prepared with acetic anhydride and pyridine the *monoacetate* (VII; R = H, R′ = Me, R″ = Ac) separated from ethyl acetate–light petroleum in needles, m. p. 198—199°, $\nu_{max.}$ 3455 (OH) and 1726 cm.$^{-1}$ (CO_2Me and OAc) [Found: C, 58·8; H, 6·6; OMe,8·7; Ac, 11·8. $C_{15}H_{18}O_5$(OMe)(OAc) requires C, 58·7; H, 6·6; OMe, 8·4; Ac, 11·7%]. Prepared from methyl dihydro-β-picrotoxinate (1 g.) by treatment with boiling 2N-sodium hydroxide (30 ml.), *dihydro-β-picrotoxinic acid* (VII; R = R′ = R″ = H) separated from ethyl acetate in prisms (0·7 g.), m. p. 249—250° (decomp.), $\nu_{max.}$ 3425, 3485, and 1736 cm.$^{-1}$ (Found: C, 57·4; H, 6·5. $C_{15}H_{20}O_7$ requires C, 57·6; H, 6·4%). Treatment of this acid with diazomethane in ether regenerated the methyl ester, m. p. and mixed m. p. 249—250°.

Oxidation of Dihydro-β-picrotoxinic Acid with Lead Dioxide.—This acid (0·4 g.) in acetic acid (4 ml.) was treated with lead dioxide (0·4 g.) at 100° for 45 min. The excess of lead dioxide was then removed by filtration and washed with acetic acid, and the combined filtrate and washings were evaporated to dryness *in vacuo*. The solid residue was dissolved in water (30 ml.), and the *ketone* (VIII) isolated by continuous extraction in ether and purified by crystallisation from ethyl acetate, giving needles (0·19 g.), m. p. 216—217°, $\lambda_{max.}$ 302 mμ (log ε, 1·46), $\nu_{max.}$ 3427 (OH), and 1767 cm.$^{-1}$ (*cyclo*pentanone) (Found: C, 62·9; H, 6·9. $C_{14}H_{18}O_5$ requires C, 63·1; H, 6·8%). The 2 : 4-*dinitrophenylhydrazone* separated from alcohol in yellow needles, m. p. 280—289° (decomp.) (Found: C, 53·9; H, 4·9; N, 12·6. $C_{20}H_{22}O_8N_4$ requires C, 53·8; H, 4·9; N, 12·6%).

neo*Picrotoxinin Acetate* (X; R = :CMe_2, R′ = Ac).—(*a*) Prepared according to the method of O'Donnell *et al.*,[8] *neo*picrotoxinin acetate separated from alcohol in colourless rod-like prisms, m. p. 189—190·5°, $\nu_{max.}$ 1795 ($\beta\gamma'$-unsaturated γ-lactone), 1773 (γ-lactone), and 1736 cm.$^{-1}$ (acetate).

(*b*) Finely powdered anhydropicrotin (5 g.), suspended in acetic anhydride (25 ml.) containing anhydrous ferric chloride (0·7 g.), was kept at room temperature until a homogeneous solution was obtained (7—14 days), which was then poured into water (200 ml.) and set aside for 2 days. The precipitated *neo*picrotoxinin acetate separated from alcohol in colourless rods (3·8 g.), m. p. and mixed m. p. 189—190·5° [infrared spectrum identical with compound derived from (*a*) above] (Found: C, 61·0; H, 5·4; Ac, 13·5. Calc. for $C_{15}H_{15}O_5$·OAc: C, 61·1; H, 5·4; Ac, 12·9%).

neo*Picrotoxinin Benzoate* (X; R = :CMe_2, R′ = Bz).—Treatment of anhydropicrotin with benzoyl chloride according to the method of Horrmann [6] gave *neo*picrotoxinin benzoate, purified by sublimation at 240°/0·01 mm., giving needles, m. p. 249—250°, $\lambda_{max.}$ 214, 274, 282 mμ (log ε 3·78, 3·07, 2·97) $\nu_{max.}$ 1808 ($\beta\gamma'$-unsaturated γ-lactone), 1785 (γ-lactone), 1724 (benzoate), 1605 and 1595 cm.$^{-1}$ (aromatic) (Found: C, 66·5; H, 5·1. Calc. for $C_{22}H_{20}O_7$: C, 66·7; H, 5·1%).

neo*Picrotoxic Acid* (XI; R = H).—Hydrolysis of either *neo*picrotoxinin acetate or the benzoate with 3% aqueous sodium hydroxide for 30 hr. at 0°, and subsequent continuous extraction with ether of the acidified hydrolysate, gave neo*picrotoxic acid*, colourless needles, m. p. 242—245° (decomp.) (from ethyl acetate–benzene), $\nu_{max.}$ 3500 and 3270 (OH), 1765 (γ-lactone), and 1712 cm.$^{-1}$ (CO_2H) (Found: C, 57·9; H, 6·0. $C_{15}H_{18}O_7$ requires C, 58·1; H, 5·8%). Prepared with diazomethane, the *methyl ester* (XI; R = Me) separated from ethyl acetate–light petroleum in plates, m. p. 253—256°, $\nu_{max.}$ 3500 (OH), 1789 (γ-lactone), and 1738 cm.$^{-1}$ (CO_2Me) (Found: C, 59·1; H, 6·3; OMe, 9·9. $C_{16}H_{20}O_7$ requires C, 59·2; H, 6·2; OMe, 9·6%). This ester dissolved readily in cold dilute sodium hydroxide and was reprecipitated unchanged on acidification. Prepared with acetic anhydride and pyridine, the *monoacetate* separated from benzene in needles, m. p. 173—174°, $\nu_{max.}$ 3497 (OH), 1792 (γ-lactone), and 1730 cm.$^{-1}$ (CO_2Me and OAc) (Found, on a sample sublimed at 165°/0·01 mm.: C, 58·3; H, 6·0; Ac, 10·7. $C_{16}H_{19}O_6$·OAc requires C, 59·0; H, 6·0; Ac, 11·7%).

Ozonolysis of neo*Picrotoxinin Acetate.*—This compound (5 g.) in ethyl acetate (150 ml.) at −80° was treated with a slow stream of ozonised oxygen for 5 hr. Removal of the solvent *in vacuo* gave the *ozonide* which separated from alcohol in colourless needles (4·0 g.), m. p. 182—184° (decomp.) (Found: C, 53·0; H, 5·0. $C_{17}H_{18}O_{10}$ requires C, 53·4; H, 4·7%). This ozonide was stable to prolonged treatment with cold water and was decomposed slowly by boiling 2N-sulphuric acid.

The ozonide (1 g.) in ethyl acetate (100 ml.) containing Adams catalyst (0·1 g.) was agitated in hydrogen until absorption ceased (20 min.) (vol. absorbed, 75 ml.; theor., 59 ml.); the catalyst was then removed and the solution extracted with water (4 × 20 ml.). The aqueous extract was distilled in a current of steam and the distillate treated with an excess of 2 : 4-dinitrophenylhydrazine sulphate in dilute sulphuric acid. The orange precipitate was collected after 24 hr. and crystallised from alcohol, to give acetone 2 : 4-dinitrophenylhydrazone in orange needles (320 mg., 52%), m. p. and mixed m. p. 125—127°. The ethyl acetate solution (after removal of acetone in water) was dried ($MgSO_4$), the solvent evaporated *in vacuo* and the residue crystallised from ethyl acetate–light petroleum, to give colourless plates (450 mg.), m. p. 180—182° (decomp.), of the ozonolysis *product*. When dried at 140°/1 mm. these plates fell to a hygroscopic powder, m. p. 223—228° (Found, for a dried sample: C, 54·3; H, 3·9. $C_{14}H_{12}O_8$ requires C, 54·5; H, 3·9%). The infrared spectrum of the hydrated material showed bands at 3570 (hydrate water), 1812 (β-keto-γ-lactone?), 1779 (γ-lactone), 1754 (OAc) and 1701 cm.$^{-1}$ (ketonic CO?). This compound was recovered unchanged after attempted formation of a 2 : 4-dinitrophenylhydrazone whereas treatment with hydroxylamine hydrochloride and pyridine at room temperature for 2 days yielded an intractable water-soluble product which gave a ruby-red ferric reaction.

Reaction of Picrotin with Acetic Anhydride.—(*a*) Picrotin (2·3 g.), which had been reprecipitated from 2N-sodium hydroxide with hydrochloric acid, was suspended in acetic anhydride (10 ml.) containing concentrated sulphuric acid (0·15 ml.) and set aside at room temperature for 2 days. The insoluble residue was collected and recrystallised from acetic acid, to give prisms (1·1 g.), m. p. 324—326° (decomp.), identified as anhydropicrotin by mixed m. p. and infrared spectrum. Decomposition of the acetic anhydride filtrate with water

(50 ml.) gave a solid (0·6 g.) which separated from alcohol in needles (0·25 g.), m. p. 215—230°, raised to 252—254° by repeated recrystallisation and identified as picrotoxinin acetate by mixed m. p. and infrared spectrum.

(*b*) A sample of picrotin (2·3 g.), which had been precipitated as in method (*a*) and then recrystallised from methanol, was treated with acetic anhydride (10 ml.) and concentrated sulphuric acid (0·15 ml.) for 2 days at room temperature. The insoluble residue was collected and crystallised from acetic acid, to give prisms (0·4 g.), m. p. 324—326°, of anhydropicrotin. The acetic anhydride filtrate was decomposed with water (50 ml.), and the resultant precipitate collected and crystallised from alcohol, to give needles (0·9 g.), m. p. 189—190·5°, identified as *neo*picrotoxinin acetate by mixed m. p. and infrared spectrum.

Treatment of Picrotin with Benzoyl Chloride.—A solution of picrotin (2 g.) in benzoyl chloride (9 ml.) was heated under reflux for 2 hr. and set aside for 24 hr. The material which separated was collected and recrystallised from acetic acid, to give prisms (350 mg.), m. p. 324—326° (decomp.), of anhydropicrotin. The benzoyl chloride solution was then added to 2N-sodium carbonate (90 ml.), and the mixture was set aside for 4 days. The brown solid which separated was collected and crystallised from alcohol, to give needles (0·8 g.), m. p. 240—246° raised to 249—250° on sublimation at 205°/0·01 mm., and identified as *neo*picrotoxinin benzoate by mixed m. p. and infrared spectrum.

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