428. *Picrotoxin and Tutin. Part VI.* Methylation and Methanolysis.*

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Methylation of picrotoxinin and related substances by methyl sulphate–alkali, methyl iodide–silver oxide, and diazomethane has been further investigated. It has been shown in particular that picrotoxinin with methanol and diazomethane gives the methyl ether of methyl picrotoxate, Sutter and Schlittler's "Compound C" (*Helv. Chim. Acta*, 1950, **33**, 902); other transformation products of "C" described by Sutter and Schlittler are identified accordingly.

The reaction of picrotoxinin and related substances with alcoholic alkoxides has been shown to proceed in the presence of only a trace of the alkoxide. The nature of the product formed may vary with the amount of alkoxide used.

In Part III (*J.*, 1949, 806) the hope was expressed that methylation would be of help in stabilising the structures of picrotoxin and related compounds. The natural division of these substances into two groups, alkali-stable and alkali-unstable (Part IV, *J.*, 1952, 1042), suggested the use of the silver oxide method for the latter and either this or the methyl sulphate method for the former. In addition, as has been shown by Sutter and Schlittler (*Helv. Chim. Acta*, 1950, **33**, 902), diazomethane has potential applications.

The parent substances picrotoxinin and picrotin (together with *neo*picrotoxinin) are not stable to alkali, but unfortunately the silver oxide method fails to methylate them, and diazomethane when it reacts (as with picrotoxinin) brings about also other changes (see below). Dihydropicrotoxinin and picrotin are moderately stable for short periods to cold dilute alkali but the former was recovered unchanged after attempted methylation with methyl sulphate and the latter gave a complex mixture. The chief alkali-stable compounds are the bromopicrotoxinic acids, bromo*neo*picrotoxinic acid, picrotoxic acid, α- and β-picrotinic acids, picrotindicarboxylic acid, and Sutter and Schlittler's "Compound C" (*Helv. Chim. Acta*, 1950, **33**, 902). Methylation of β-bromopicrotoxinic acid with methyl sulphate was described in Part III (*loc. cit.*) but the corresponding reaction with bromo*neo*picrotoxinic acid could not be realized. Picrotoxic and α-picrotinic acids and their esters, however, react smoothly to give monomethyl ethers.

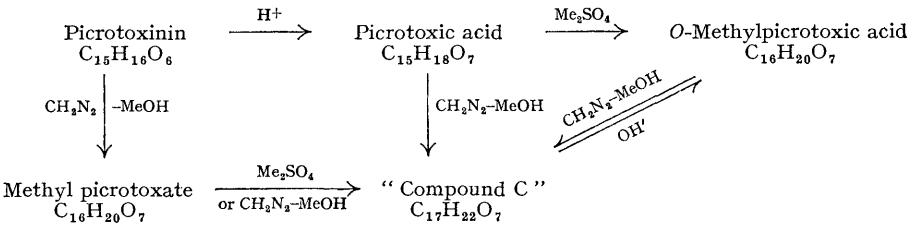
The silver oxide method has been reported to have some success when used under vigorous conditions (Mercer and Robertson, *J.*, 1936, 288; Sutter and Schlittler, *loc. cit.*) but under normal conditions we have not achieved useful results, β-picrotinic acid, for example, merely giving its methyl ester.

The use of diazomethane has hitherto been complicated by the somewhat uncertain nature of the reactions involved (Sutter and Schlittler, *loc. cit.*). It is also not clear from the previous work whether the changes observed are due exclusively to the diazomethane, or to alkali added to catalyse the reaction, or even to the traces of alkali present in preparations of diazomethane which have not been distilled. We therefore re-investigated the reactions described by Sutter and Schlittler; in the main we confirm their experimental observations, but we re-interpret certain of them and correlate the substances described with well-known derivatives of picrotoxin.

In the presence of added potassium hydroxide, picrotoxinin reacted with methyl alcohol and ethereal diazomethane to give mainly "Compound C," as already described, together with a small amount of dimethyl picrotoxinindicarboxylate. With no added potassium hydroxide, and with an excess of undistilled diazomethane, the reaction led to approximately equal amounts of "C" and the ester. By use of distilled ethereal diazomethane, "Compound C" was formed practically exclusively. This variation is explained below. Sutter and Schlittler mention the formation of a certain amount of dimethyl picrotoxinindicarboxylate in this reaction; the properties of our product agree with the

* Part V, *J.*, 1952, 1597.

data of Horrmann (*Annalen*, 1916, **411**, 273) and the positive response to periodic acid agrees with the observations of Conroy (*J. Amer. Chem. Soc.*, 1951, **73**, 1889). The nature of " Compound C " was discussed inconclusively by Sutter and Schlittler; in Part IV of this series a possible way in which " Compound C " could be simply derived from picrotoxinin was suggested. It has now been identified as the methyl ether of methyl picrotoxate by the reactions shown in the annexed scheme.



The other main transformation products of " C " described by Sutter and Schlittler are identified as follows: " E " is *O*-methylpicrotoxic acid; " H " is the (known) *O*-methylpicrotoxonic acid (Horrmann and Wachter, *Ber.*, 1916, **49**, 1554); " P " is the (known) methyl *O*-methylpicrotoxonate (*idem, ibid.*). The differences in the reported melting points of " H " and " P " are of no significance, as they depend on the degree of preheating and rate of heating. The melting point of " E," however, is given as 229·5—230° whereas that of the product of methylation of picrotoxic acid with methyl sulphate and alkali we found to be 215°. We therefore repeated the preparation of " E " by hydrolysis of " C " and in different preparations obtained only material varying in melting point from 215° to 220°, indistinguishable from that obtained from picrotoxic acid.

In one experiment where diazomethane reacted with picrotoxinin we isolated some methyl picrotoxate, along with its methyl ether and dimethyl picrotoxinindicarboxylate. As this on further treatment with diazomethane gives the methyl ether we conclude that methyl picrotoxate is an intermediate stage in the transformation of picrotoxinin into " C."

In agreement with Sutter and Schlittler we found no reaction between picrotin and diazomethane, except in the presence of added alkali, which led merely to methanolysis.

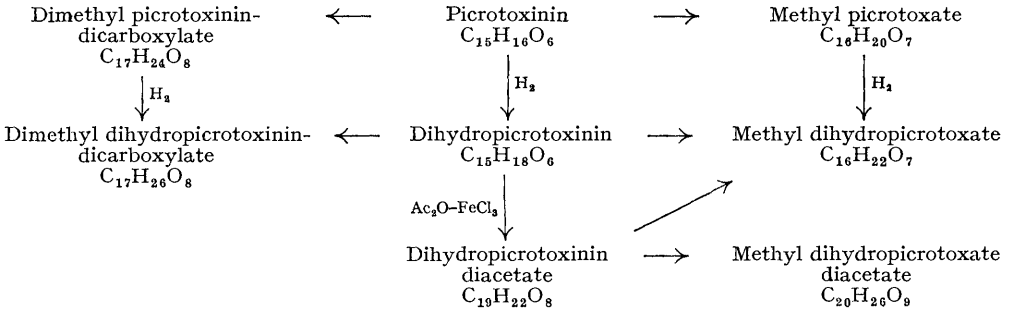
*neo*Picrotoxinin, although originally obtained from picrotoxinin, is more closely related to picrotin (Slater and Wilson, *Nature*, 1951, **107**, 324), from which it may be obtained by dehydration, and in conformity with this view it does not react with diazomethane. α-Picrotinic acid, although obtained from picrotin, is also related to picrotoxinin through its dehydration to picrotoxic acid, and it is not therefore immediately obvious to which series it is most closely allied. That it is actually a member of the picrotoxinin series is shown by its reaction with diazomethane, giving the methyl ester of the methyl ether, also obtained by use of methyl sulphate. β-Picrotinic acid on the other hand simply gives its ester when treated with diazomethane (cf. α-picrotoxinic acid).

Bromo*neo*picrotoxinin (lactone) has previously been shown to be extremely readily but reversibly convertible into bromo*neo*picrotoxinic acid (hydroxy-acid). This acid and diazomethane merely gave bromo*neo*picrotoxinin. As nitrogen was rapidly eliminated during the initial reaction the methyl ester may well be formed initially and lactonise during working up. A similar explanation may account for the peculiar nature of the reaction product, referred to above, obtained when picrotin is methylated with methyl sulphate. The only well-defined homogeneous material isolated was a stable methyl derivative, $C_{16}H_{20}O_7$. Accompanying this substance is other material from which by fractionation may be isolated various somewhat ill-defined specimens some of which show the interesting property of reverting readily to picrotin. They may represent impure specimens of methyl esters of the mono- or di-basic acids which are formed when picrotin dissolves in cold dilute alkali but on acidification immediately re-form picrotin.

The reaction between α-picrotoxinic acid and diazomethane has been reported to yield picrotoxinindicarboxylic ester (Sutter and Schlittler, *loc. cit.*) but the reaction was carried out in the presence of alkali and methanol and hence cannot be interpreted unequivocally. We have found that the prolonged action of distilled ethereal diazomethane on a methanolic

solution of α-picrotoxinic acid gives only the methyl ester. The formation of the dicarboxylic ester can be ascribed therefore to the action of the alkali. The reaction is of importance for the relation between picrotoxinin and α-picrotoxinic acid (cf. Part IV, *loc. cit.*).

The reaction of picrotin and picrotoxinin with alcoholic solutions of alkoxides has been examined by Horrmann (*Annalen*, 1916, **411**, 273). Picrotin and methanolic sodium methoxide (1 mol.) gave a mixture of potassium β-picrotinate and methyl α-picrotinate. Picrotoxinin somewhat similarly gave potassium methyl picrotoxinindicarboxylate and methyl picrotoxate. We have further examined this method of opening the lactone systems, using mainly a trans-esterification technique (excess of alcohol and a catalytic amount of alkoxide). Under these conditions ring-fission occurs readily at room temperatures; hence, for reactions between picrotoxin derivatives and diazomethane in methanol–ether alkali should be excluded. The results of trans-esterification also emphasize the difference between picrotin and picrotoxinin. With picrotin, the use of a catalytic amount of sodium methoxide gives essentially the same products as are obtained with a molar proportion, *viz.*: methyl α- and β-picrotinate in approximately the same proportions. However, picrotoxinin in presence of 0·05—1 mole of sodium methoxide gives mainly methyl picrotoxate, but in presence of 0·01 mole gives mainly dimethyl picrotoxinindicarboxylate. The dihydro-derivative of the latter is identical with the main product of the methanolysis of dihydropicrotoxinin in the presence of about 0·02 mole of sodium methoxide. A by-product of the last reaction is methyl dihydropicrotoxate which is also formed by complete methanolysis of dihydropicrotoxinin diacetate (Part IV). By varying the reaction conditions this diacetate can be transformed into methyl dihydropicrotoxate diacetate. These inter-relations are summarized in the annexed scheme, where each reaction is effected by methanolic sodium methoxide, except as shown.



It is now possible to interpret the variations in the reaction between picrotoxinin and diazomethane. In general, except where distilled ethereal diazomethane is used, two main reactions are involved: that between picrotoxinin and methanolic diazomethane, which leads first to methyl picrotoxate, and then to its methyl ether (" Substance C "), and methanolysis of picrotoxinin by sodium methoxide. The last reaction is itself composite and may either reinforce or oppose the formation of methyl *O*-methylpicrotoxate: one, leading to dimethyl picrotoxinindicarboxylate, proceeds at a rate little dependent on the amount of sodium methoxide present, but the rate of the other, leading to methyl picrotoxate, is dependent thereon. The reaction between picrotoxinin and methanolic diazomethane in the complete absence of methoxide yields only methyl *O*-methylpicrotoxate. In the presence of a relatively large amount of methoxide the formation of this takes place by two routes—directly by the action of methanolic diazomethane, and indirectly by the action of methanolic sodium methoxide, by way of methyl picrotoxate which is then attacked by diazomethane. The amount of dimethyl picrotoxinindicarboxylate formed by methanolysis, as seen from the separate experiments, will be small and the product of the overall reaction is again substantially methyl *O*-methylpicrotoxate. In the presence of a trace only of methoxide, however, methanolysis gives almost exclusively dimethyl picrotoxinindicarboxylate while the diazomethane reaction takes

its normal course. The product of the overall reaction is therefore a mixture of approximately equal amounts of methyl *O*-methylpicrotoxate and dimethyl picrotoxinin-dicarboxylate.

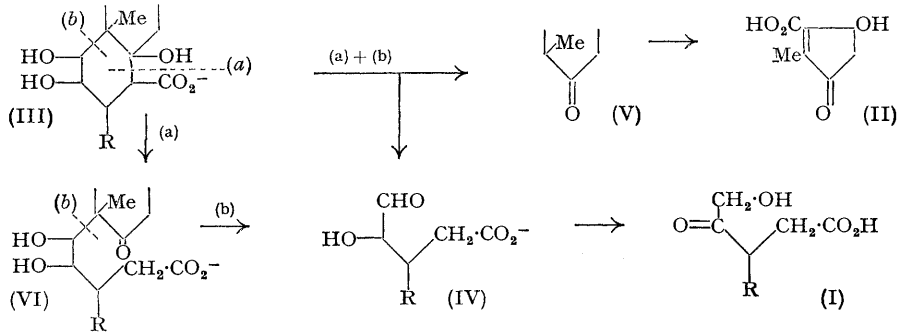
The formation of methyl ethers from picrotoxic acid and α-picrotinic acid under the influence of diazomethane suggests that they are α-hydroxy-acids (cf. Part IV) and this view provides a ready explanation of the course of the thermal decomposition of α-picrotinic acid to picrotoxic acid and picrotin lactone (Horrmann *et al.*, *Ber.*, 1912, **45**, 3080; *Annalen*, 1916, **411**, 273). Picrotoxic acid is produced merely by dehydration, the hydroxyl group lost being that of the dimethylcarbinol group and not that present in the position α to the carboxyl group. Picrotin lactone has hitherto been regarded as isomeric with picrotoxic acid but its high melting point and low solubility even in dioxan (which in our experience readily dissolves all " normal " compounds in this series) suggest a much higher molecular weight. Bimolecular elimination of water is a well-known characteristic of α-hydroxy-acids, and picrotin lactone is probably a C_{30} rather than a C_{15} compound.

Methanolysis of β-bromopicrotoxinin readily yielded methyl β-bromopicrotoxinate. Conversion of bromopicrotoxinin into this ester by diazomethane (Sutter and Schlittler, *loc. cit.*) was carried out in the presence of methanol and added alkali, so that again it is impossible to decide whether the product was really formed by the diazomethane. When β-bromopicrotoxinin is treated with distilled ethereal diazomethane in the presence of methanol slow reaction does in fact take place with the formation of methyl β-bromo-picrotoxinate.

In studying the chemistry of picrotin and picrotoxinin it has proved difficult to correlate completely the many known transformation products. There are, however, several frequently observed reactions which probably depend on specific structural features and may therefore be used as a guide in following the transformations. The most striking of these are: (1) formation of a saturated, relatively insoluble monobromo-substitution product on treatment of an unsaturated compound with bromine water; (2) fission by heating with alkali; and (3) reaction with diazomethane, leading to the methylation of a non-acidic hydroxyl group. As will be seen from what follows, these reactions are not unrelated.

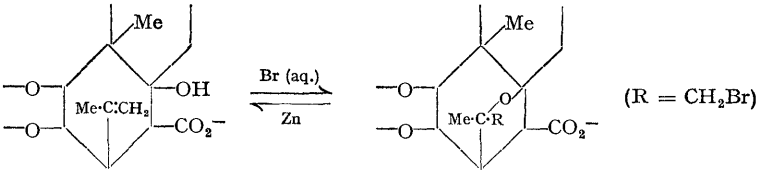
Although the course of (1) is still not completely established it is clear that the mere presence of a double bond is insufficient, since neither picrotoxic acid nor its methyl ester methyl ether reacts thus.

We ascribe reaction (3) to the presence, in the position α to a hydroxyl group, of an activating group such as an ester grouping (actual or potential).

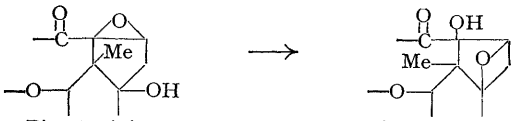


With regard to (2) it has been suggested (Schlittler and Sutter, 1st Internat. Congr. Biochemistry, Cambridge, 1949; Conroy, *loc. cit.*) that alkaline fission is the result of a reversed aldol reaction. If the five-membered ring of (II) is assumed to be present in the various precursors of this substance, then the essential overall change is (III) ⟶ (IV) + (V), followed by (IV) ⟶ (I) and (V) ⟶ (II). While fission at (*a*) can be viewed as independent of that at (*b*), the reverse is not true—fission at (*b*) depends on the formation of a carbonyl group, as in (VI), which can provide the necessary activation. If this view is correct then any compound in which the tertiary alcohol group of (III) is

absent will be stable to alkali. This absence may conceivably be absolute or relative, *i.e.*, oxygen may be completely absent or the alcohol group may be converted into an alkali-stable group OR. In conformity with these views two groups of alkali-stable compounds appear to exist. The various saturated monobromo-derivatives referred to under (1) are alkali-stable but on debromination give rise to products (picrotoxinin, α-picrotoxinic acid, *neo*picrotoxinin) which undergo fission with alkali. This relative stability is adequately explained by the mechanism for the bromination reaction proposed by O'Donnell and Robertson (*J.*, 1939, 1261) if the hydrogen eliminated as hydrogen bromide comes from the tertiary hydroxyl group :



The alkali-stability of picrotoxic acid and its derivatives, which is accompanied by failure to undergo the bromination reaction, shows clearly on both grounds that the tertiary hydroxyl group is absent. Two explanations suggest themselves. If Conroy's formula for picrotoxinin is accepted, then the position of the ether linkage in picrotoxic acid may be different from that in picrotoxinin itself, leading to some such partial formulation as :



Picrotoxinin Picrotoxic acid

We have already questioned the validity of the assumption of the presence of an ether linkage in picrotoxinin and until more positive evidence for its presence is forthcoming prefer to assume its absence. Bromo*neo*picrotoxinin can be converted into a monoacetate and, if the formation of the bromo-compound initially involves loss of hydrogen from one hydroxyl group, then *neo*picrotoxinin, and thus presumably picrotoxinin, must contain two hydroxyl groups. The absence of an ether linkage in picrotoxic acid would require its stability to alkali to be due to an absolute absence of the tertiary hydroxyl group. This view, that picrotoxic acid is fundamentally different in structure from picrotoxinin, is in conformity with the behaviour of its derivatives, dihydropicrotoxic acid and methyl *O*-methylpicrotoxate. Methyl dihydropicrotoxate is recovered unchanged after evaporation to dryness with boiling concentrated nitric acid and after oxidation at room temperature with alkaline permanganate. Attempts to degrade methyl *O*-methylpicrotoxate to the aromatic state by zinc dust or selenium resulted in the recovery of unchanged material (*e.g.*, with selenium at 260° or on distillation with zinc dust) or, at higher temperatures (*e.g.*, with selenium at 300°), complete charring.

Experimental

Methylation of Picrotin.—Picrotin (1 g.) in dilute aqueous sodium hydroxide was methylated with methyl sulphate (2 g.). Further alkali was added as required and the mixture was warmed gently. A little concentrated hydrochloric acid was then added, precipitating a gummy material (A). The mother-liquor gradually deposited solid material (B). Fractions (A) and (B) from several preparations were combined and subjected to exhaustive fractional crystallisation from aqueous methanol or water. (A), which slowly solidified, thus gave *methylpicrotin* of m. p. 210°, raised by chromatography (alumina; dioxan) to 215° (Found : C, 59·0; H, 5·9; MeO, 7·8. $C_{16}H_{20}O_7$ requires C, 59·3; H, 6·2; MeO, 9·7%). It is remarkably stable to heat. Unlike picrotin, it is not readily soluble in dilute aqueous sodium hydroxide. The mother-liquors yielded a little more of this material and then a succession of fractions characterized by the fact that when heated from room temperature they showed high, though somewhat indefinite, m. p.s (>200°) but when placed in a bath pre-heated to a lower temperature (*e.g.*, 170°) melted immediately. *E.g.*, the fraction immediately following the above two melted at

ca. 230—235° after much previous softening (Found : C, 53·4, 53·7; H, 6·35, 6·14; MeO, 1·9. Found, in material dried over P_2O_5 at 100°/20 mm. : C, 58·5; H, 6·1. Calc. for $C_{15}H_{18}O_7$: C, 58·1; H, 5·8%). After being heated somewhat, then recrystallised, it melted at approximately the same temperature but no longer showed the low m. p. from a preheated bath. It did not depress the m. p. of picrotin. When the original material is taken up in dilute aqueous sodium hydroxide and then reprecipitated with hydrochloric acid, it is usually recovered as picrotin. Fraction (B) yielded some picrotin and various fractions showing in general qualitative behaviour similar to that just described.

O-*Methylpicrotoxic Acid.*—Picrotoxic acid (1 g.) was methylated as above in 2N-sodium hydroxide solution by methyl sulphate with gentle warming. The product obtained on acidification was crystallised from water, treated as the sodium salt with charcoal, and finally recrystallised, to give the *methyl ether monohydrate* (Found : C, 56·3; H, 6·4; MeO, 8·9. $C_{16}H_{20}O_7,H_2O$ requires C, 56·1; H, 6·4; 1MeO, 9·1%). It melted at *ca.* 215° when not placed in a preheated bath. If placed in a bath heated to, *e.g.*, 170° the substance melted.

Methylation of β-*Picrotinic Acid.*—(*a*) β-Picrotinic acid (1 g.) was heated under reflux with methyl iodide (5 g.) and silver oxide (3·4 g.) in absolute methanol (20 ml.) for 2 hours. The solution was filtered and evaporated and the residue crystallised from water gave the ester (0·6 g.), m. p. 231° (Horrmann, *loc. cit.*, gives m. p. 231°) (Found : C, 55·8; H, 6·7; MeO, 9·3. Calc. for $C_{16}H_{22}O_8$: C, 56·1; H, 6·4; 1MeO, 9·1%). This m. p. is low (cf. below).

(*b*) β-Picrotinic acid was dissolved in methanol and to it was added undistilled ethereal diazomethane. There was an immediate dense precipitation of a white solid which was filtered off and crystallised from water from which it separated very slowly in small granular crystals, m. p. *ca.* 220° after some previous softening. Recrystallization finally gave material, m. p. *ca.* 240—242°, showing no depression with the above ester.

Reaction of Picrotoxinin with Diazomethane.—(a) *In the presence of potassium hydroxide.* Picrotoxinin (2 g.) in excess of methanol was treated with ethereal diazomethane in the presence of a little aqueous potassium hydroxide as described by Sutter and Schlittler (*loc. cit.*). Crystallisation of the product from methanol gave methyl *O*-methylpicrotoxate (1·2 g.). The mother-liquor yielded further amounts of this ester and a small amount of material, m. p. 185° after previous softening alone or mixed with material described below.

(b) *In the absence of potassium hydroxide.* Picrotoxinin (1 g.) in methanol (20 ml.) was treated from time to time with undistilled ethereal diazomethane until reaction appeared to be complete. The solvent was removed and the residue crystallised from methanol, to give a mixture of crystals which was separated by hand into needles and hard plates. The needles on recrystallisation yielded methyl *O*-methylpicrotoxate, m. p. 176° (120 mg.). The plates on recrystallisation gave no material of sharp m. p. although there was evidence of a constituent of m. p. above 180°. All the material, with the exception of the pure methyl *O*-methylpicrotoxate, was combined, and chromatographed in dioxan on alumina. The first eluates were rich in the above ester but the last fractions gave dimethyl picrotoxinindicarboxylate, m. p. 187° (Found : C, 57·0; H, 6·6. Calc. for $C_{17}H_{24}O_8$: C, 57·3; H, 6·8%). Further separation is possible but tedious. The two compounds appear to be formed in approximately equal quantities. In one preparation the final eluates gave methyl picrotoxate (0·1 g. from 2 g. of picrotoxinin), m. p. 171·5° alone and mixed with the ester obtained by methanolysis (see below).

Reaction of Picrotoxic Acid with Diazomethane.—Picrotoxic acid (1 g.) in methanol (20 ml.) with undistilled ethereal diazomethane gave a high yield of methyl *O*-methylpicrotoxate, m. p. and mixed m. p. 176° (Found : C, 60·5; H, 6·4; MeO, 16·9. Calc. for $C_{17}H_{22}O_7$: C, 60·4; H, 6·5; 2MeO, 18·1%). The same product was obtained by using distilled diazomethane, and chromatographic analysis of the total product failed to reveal the presence of another component.

Methyl O-*Methylpicrotoxate.*—(*a*) *O*-Methylpicrotoxic acid and ethereal diazomethane gave the ester, m. p. 175—177° (from methanol). (*b*) Methyl picrotoxate (450 mg.), methyl sulphate (1 g.), and alkali gave the same product (0·3 g.), m. p. 174° (from aqueous methanol). (*c*) Methyl picrotoxate in methanol, with an excess of ethereal diazomethane, gave the ester, m. p. 175° (from methanol). None of the above preparations showed any depression of m. p. with that obtained from picrotoxinin and diazomethane.

Methyl O-*Methyl-*α-*picrotinate.*—(*a*) Methyl α-picrotinate (1 g.), in the minimum quantity of 2N-sodium hydroxide, and methyl sulphate (2 g.) as above gave, after acidification, concentration, and storage, hard lustrous cubes. The *ether*, crystallised from water, had m. p. 182° (0·6 g.) (Found : C, 57·8; H, 6·6; MeO, 15·6. $C_{17}H_{24}O_8$ requires C, 57·3; H, 6·8; 2MeO, 17·4%). It is soluble in dilute aqueous sodium hydroxide and precipitated unchanged on

acidification, as is the case with methyl α-picrotinate, methyl picrotoxate, and methyl *O*-methylpicrotoxate. (*b*) Methyl α-picrotinate (300 mg.) in methanol (10 ml.) was treated with ethereal diazomethane until a permanent colour remained for several hours. The solution was reduced in bulk and gradually large crystals of the methyl ether separated. Recrystallisation from water gave hard thick plates (230 mg.), m. p. 178°, showing no mixed m. p. depression with the above ether (Found : C, 58·0; H, 7·2; MeO, 15·8%).

Reaction between Bromoneopicrotoxinic Acid and Diazomethane.—Bromo*neo*picrotoxinic acid (170 mg.) in methanol (10 ml.) and excess of ethereal diazomethane gave bromo*neo*picrotoxinin, m. p. and mixed m. p. 274° (decomp.) (from alcohol) (Found : C, 48·5; H, 4·5. $C_{15}H_{15}O_6Br$ requires C, 48·5; H, 4·1%).

Reaction between α-Picrotoxinic Acid and Diazomethane.—A solution of α-picrotoxinic acid in methanol, treated with ethereal diazomethane (distilled or undistilled), gave a product which melted, after crystallisation from aqueous methanol, at 183—184° (Found : C, 59·0; H, 6·15; MeO, 9·5. Calc. for $C_{16}H_{20}O_7$: C, 59·3; H, 6·2; 1MeO, 9·6%). This showed no m. p. depression with methyl α-picrotoxinate prepared by debromination of methyl β-bromopicrotoxinate.

Methanolysis of Picrotin.—Picrotin (1 g.) was dissolved in methanol (20 ml.), 2 drops of sodium methoxide solution (1 g. sodium in 25 ml. methanol) were added, and the solution was set aside for 12 hours. Large prisms of methyl β-picrotinate crystallised, having m. p. 234—236° alone or mixed with specimens prepared by the alternative routes. The mother-liquor yielded further amounts, the total yield being 0·58 g. Recrystallised from alcohol, the ester had m. p. 238° (Found : C, 55·8; H, 6·4. Calc. for $C_{16}H_{22}O_8$: C, 56·1; H, 6·4%).

An aqueous extract of the final residue from the mother-liquor, when kept in a vacuum-desiccator, deposited a white solid (0·4 g.), m. p. 239°. This product showed no m. p. depression with methyl α-picrotinate, m. p. 239°, prepared by Horrmann's method, but a marked depression with methyl β-picrotinate.

Methanolysis of Picrotoxinin.—(*a*) Picrotoxinin (1 g.) was dissolved in methanol (40 ml.), and sodium (5 mg.) was added. After a day the solution was acidified with acetic acid and evaporated to dryness under reduced pressure. The residue crystallised from water as fine white needles (0·8 g.), m. p. 170°, undepressed on admixture with methyl picrotoxate. (*b*) Picrotoxinin (500 mg.) was dissolved in methanol (20 ml.) and one drop of a solution of sodium methoxide (1 g. of sodium in 25 ml. of methanol) was added. After a day, the solution was acidified with a drop of acetic acid and evaporated to small volume. Addition of a few drops of water caused a copious precipitate which, crystallised from water, had m. p. 188° (310 mg.) (Found : C, 57·0; H, 6·6. Calc. for $C_{17}H_{24}O_8$: C, 57·3; H, 6·7%). The product showed no depression in m. p. when mixed with the substance of similar m. p. obtained by the action of diazomethane on picrotoxinin. The *diacetate* was prepared by heating the product (400 mg.) with acetic anhydride (14 ml.) for 2 hours, pouring the whole into water (10 ml.), and evaporating the resulting solution to dryness. The residue, crystallised from methanol, had m. p. 134° (300 mg.) (Found : C, 57·2; H, 6·3; AcO, 20·3; MeO, 14·2. $C_{21}H_{28}O_{10}$ requires C, 57·3; H, 6·36; 2AcO, 19·5; 2MeO, 14·1%). The dihydro-derivative was prepared by hydrogenating the compound (200 mg.) in alcohol (15 ml.) containing concentrated hydrochloric acid (1 drop) and water (2 ml.) in the presence of Adams's catalyst. Uptake of hydrogen (uncorr.) was 29 c.c. The product, crystallised from water, melted at 178°.

Methanolysis of Dihydropicrotoxinin.—(*a*) Methanolysis of dihydropicrotoxinin (300 mg.) in methanol (20 ml.) to which was added one drop of sodium methoxide solution (1 g. of sodium in 25 ml. of methanol) yielded a product which on crystallisation from water melted at 178° (yield, 180 mg.) and showed no mixed m. p. depression with the above dihydro-derivative. The aqueous mother-liquors, when kept for several days in a vacuum-desiccator, deposited heavy white needles, m. p. 210° (40 mg.), showing no m. p. depression with methyl dihydropicrotoxate (see below) or with the product of methanolysis of the diacetate of dihydropicrotoxinin (see below). (*b*) Dihydropicrotoxinin (1 g.), methanol (40 ml.), and sodium methoxide (from 5 mg. of sodium), after being kept overnight and worked up in the usual way, yielded methyl dihydropicrotoxate, m. p. 210° (750 mg.), showing no depression with the material described below.

Methyl Dihydropicrotoxate.—Hydrogenation of methyl picrotoxate (180 mg.) in alcohol in the presence of Adams's catalyst resulted in the rapid absorption of hydrogen (20 c.c., at room temp. and pressure) to give the saturated ester (120 mg.), m. p. 210° (from water).

Methanolysis of Dihydropicrotoxinin Diacetate.—(*a*) Dihydropicrotoxinin diacetate, dissolved in methanol and treated with a drop of sodium methoxide solution, gave after being worked up in the usual way a *substance* which, crystallised from water, had m. p. 222° (Found : C 58·3; H, 6·45. $C_{20}H_{26}O_9$ requires C, 58·5; H, 6·3%). (*b*) The diacetate (500 mg.) in methanol (30 c.c.)

was treated with sodium methoxide (1·5 c.c. of a solution of sodium, 1 g., in methanol, 25 c.c.) and set aside overnight. Worked up in the usual way the product (methyl dihydropicrotoxate) crystallised from water in needles, m. p. 211° (Found : C, 58·6; H, 6·7; MeO, 9·3; AcO, nil. Calc. for $C_{16}H_{22}O_7$: C, 58·9; H, 6·75; 1MeO, 9·5%).

Methanolysis of Bromopicrotoxinin.—β-Bromopicrotoxinin (1 g.) in methanol was treated with a drop of sodium methoxide solution; working up in the usual way gave a product, m. p. 225° (from methanol) alone or mixed with methyl β-bromopicrotoxinate prepared from the acid by methanol–sulphuric acid or diazomethane.

Reaction of Bromopicrotoxinin with Diazomethane.—β-Bromopicrotoxinin (4 g.) in excess of methanol containing some dioxan was treated with distilled ethereal diazomethane until reaction was complete and then worked up in the usual way, to give a product (3·37 g.; m. p. 224°) showing no mixed m. p. depression with the above ester. The debrominated ester melted at 184° and the bromo-acid at 247°.

Bromoneopicrotoxinin Acetate.—Bromo*neo*picrotoxinin (70 mg.) was set aside overnight with acetic anhydride (8 ml.) and pyridine (1 ml.) and then refluxed 10 minutes. Worked up in the usual way and finally crystallised from alcohol, the *acetate* had m. p. 270° (decomp.) (Found : C, 48·5; H, 4·3; AcO, 10·3. $C_{17}H_{17}O_7Br$ requires C, 49·4; H, 4·1; AcO, 10·4%). In two further independent preparations low values for carbon were again obtained (48·4; 48·1%).

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