# 174. *Picrotoxinin and Tutin. Part III. The Hydrogenation of Picrotoxinin.*

By S. N. Slater.

By treatment with bromine water of the reaction product obtained in the hydrogenation of picrotoxinin with palladium catalysts in the presence of acid, bromo*neo*picrotoxinin is formed which can be reduced to *neo*picrotoxinin, $C_{15}H_{16}O_6$, an isomeride of picrotoxinin. It is the hitherto postulated "picrotonol precursor," its acetyl derivative being identical with the acetyl derivative of the "picrotonol precursor." Dihydro*neo*picrotoxinin is identical with "β-dihydropicrotoxinin." The relationship between picrotoxinin and *neo*picrotoxinin is discussed. The lactone system of bromo*neo*picrotoxinin is unique in the picrotoxinin series in that it may be reversibly opened. The methylation of β-bromopicrotoxinic acid is described.

The hydrogenation of picrotoxinin in the presence of palladium has been investigated by Mercer and Robertson (*J.*, 1936, 288) and by O'Donnell, Robertson, and Harland (*J.*, 1939, 1261), who have shown that the reaction is a complex one, leading (in neutral solution) to the formation of a mixture which on hydrolysis with 5% sulphuric acid yields β-dihydropicrotoxinin, dihydropicrotoxic acid, and picrotonol. The last two products are believed to arise from the action of the acid upon α-dihydropicrotoxinin and a "picrotonol precursor," respectively, and it is suggested that the picrotonol precursor may be an isomeride of picrotoxinin formed under the conditions of hydrogenation. The picrotonol precursor itself could not be isolated, but it was possible to obtain an acetyl derivative, presumably derived from it.

In Part I of this series (*J.*, 1943, 50) the author showed that tutin, $C_{15}H_{18}O_6$, the poisonous principle of the coriaria species of New Zealand, on hydrogenation in the presence of palladium and subsequent treatment with bromine water, gave a substance, believed at first to be a bromohydrotutin, $C_{15}H_{19}O_6Br$, but later (Part II, *ibid.*, p. 143) regarded as possessing the formula $C_{15}H_{17}O_6Br$. Similar results were obtained by hydrogenating picrotoxin and treating the product with bromine water, whereby a substance $C_{15}H_{15}O_6Br$ ("bromohydropicrotoxinin") was produced. It was suggested that these bromo-derivatives might have arisen from isomerisation products of tutin and picrotoxinin, formed under the influence of the catalyst and hydrogen. Attempts to repeat this work failed in the first instance.

A further investigation of the hydrogenation of picrotoxin and picrotoxinin in ethyl-alcoholic solution in the presence of a palladium–charcoal catalyst has provided an explanation of the discordant results and has led to the isolation of the previously postulated isomeride of picrotoxinin, the picrotonol precursor. If the hydrogenation is prolonged over several days the initial rapid uptake of hydrogen is succeeded by a slow absorption, considerably in excess of that required to saturate one double bond, and the final product does not react with bromine water. On the other hand, if the hydrogenation is stopped after the first rapid absorption of hydrogen is complete then the product (from either picrotoxin or picrotoxinin) reacts readily with bromine water to give the substance $C_{15}H_{15}O_6Br$, now named bromo*neo*picrotoxinin. This may be debrominated with zinc to yield an isomeride of picrotoxinin, $C_{15}H_{16}O_6$, for which the name neo*picrotoxinin* is suggested. It is a crystalline, optically active compound, similar

in its general behaviour to picrotoxinin. For instance, addition of bromine water to its hot aqueous solution precipitates bromo*neo*picrotoxinin, although only one modification is obtained in contrast to the α- and β-modifications of bromopicrotoxinin. On heating, it reduces both Fehling's solution and ammoniacal silver nitrate. It is soluble in alkali but is not reprecipitated on acidification. Its ultra-violet spectrum shows only end absorption.

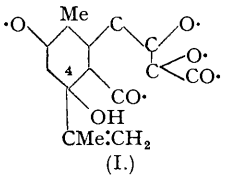
At this stage it appeared likely that *neo*picrotoxinin would prove identical with the picrotonol precursor, and confirmation of this view was found in its conversion into acetyl*neo*picrotoxinin, $C_{17}H_{18}O_7$, identical with a specimen of the acetyl derivative of the picrotonol precursor, prepared as described by O'Donnell, Robertson, and Harland (*loc. cit.*). That *neo*picrotoxinin was present in the particular specimen of crude hydrogenated picrotoxinin from which Robertson and his co-workers' acetate was prepared was shown by bromination of a portion of the material to give bromo*neo*picrotoxinin. The fact that β-dihydropicrotoxinin, like *neo*picrotoxinin, can be acetylated whilst picrotoxinin and α-dihydropicrotoxinin cannot, made it likely also that β-dihydropicrotoxinin was derived from *neo*picrotoxinin rather than picrotoxinin, and this was confirmed by hydrogenating *neo*picrotoxinin to *dihydroneopicrotoxinin*, $C_{15}H_{18}O_6$, which proved to be identical with a specimen of β-dihydropicrotoxinin prepared by the method of Robertson *et al.* In carrying out this hydrogenation it was considered desirable to use a platinum catalyst in acetic acid solution, since these conditions do not lead to secondary changes during the hydrogenation of picrotoxinin, but when this was attempted it was found that although hydrogen was absorbed the only pure material isolated from the reaction mixture was unchanged *neo*picrotoxinin. The same effect was observed in an attempt to hydrogenate acetyl*neo*picrotoxinin: by use of a palladium–charcoal catalyst the expected dihydro-derivative was obtained. The yield, however, is poor, and the reaction is clearly complex since the absorption of hydrogen is much in excess of that required for simple saturation of the double bond.

In the further study of the formation of *neo*picrotoxinin from picrotoxinin constant use was made of the characteristic formation of the readily recognisable bromo*neo*picrotoxinin, which simplified the otherwise difficult task of establishing the presence or absence of the isomeride in complex reaction mixtures. This isomerisation is dependent upon three factors: the use of palladium, the presence of hydrogen, and the presence of acid. The fact that no isomerisation occurs in the absence of hydrogen was observed by O'Donnell, Robertson, and Harland (*loc. cit.*) and this has been confirmed. This limits the yield of *neo*picrotoxinin, since hydrogenation invariably accompanies isomerisation. In an attempt to overcome this difficulty the reaction was carried out in an initially attenuated atmosphere of hydrogen, but without any improvement in the yield. The importance of the acid was shown by the use of neutral or slightly alkaline catalysts of proved activity in other hydrogenations or when used with picrotoxinin in acid solution. Thus, using palladium–barium sulphate, the absorption of hydrogen was negligible. With Raney nickel, a very slow absorption of hydrogen took place but the product on brominaton yielded only bromopicrotoxinin. Palladium–charcoal, as ordinarily prepared, is always acid in reaction unless special precautions are taken in the final stages. If such a catalyst is neutralised and used in the hydrogenation of picrotoxinin, the uptake of hydrogen is again slow and bromination of the product gives only bromopicrotoxinin. The residual acidity of palladium–charcoal catalysts is evidently sufficient, when working in otherwise " neutral " solutions (*e.g.*, hydrogenation in alcohol), to bring about the isomerisation.

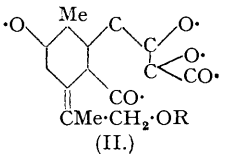
In considering the possible relationship between picrotoxinin and *neo*picrotoxinin the following five observations appear to be of importance: (*a*) The transformation takes place only in the presence of palladium, hydrogen, and acid. (*b*) Whereas picrotoxinin does not give an acetyl derivative, *neo*picrotoxinin can be acetylated, suggesting (O'Donnell, Robertson, and Harland, *loc. cit.*) a change in function of the hydroxyl group from tertiary to primary or secondary. (*c*) Whereas bromination of picrotoxinin leads to a mixture of two (stereo-?) isomeric monobromo-substitution products, bromination of *neo*picrotoxinin gives only one monobromo-substitution product. The rapid hydrogenation of picrotoxinin in acetic acid solution in the presence of a platinum catalyst may likewise be contrasted with the great resistance of *neo*picrotoxinin to hydrogenation under similar conditions. These two observations suggest a change in the molecular environment of the double bond. (*d*) As is shown below, bromo*neo*picrotoxinin differs strikingly from the bromopicrotoxinins in its behaviour with alkali, since the opening of the lactone system in this case is reversible. (*e*) When boiled with dilute mineral acid picrotoxinin gives the hydroaromatic picrotoxic acid but *neo*picrotoxinin gives the aromatic picrotonal.

Although the full significance of (*a*) is not yet apparent, (*b*) and (*c*) suggest some sort of allyl-

like rearrangement. Employing the partial formula for picrotoxinin developed by Harland and Robertson (*J.*, 1939, 937) and placing the tertiary hydroxyl group at $C_4$, the allyl position with respect to the double bond, the partial formula (I) is obtained which by allyl rearrangement would give (II; R = H) for *neo*picrotoxinin. Such further evidence as has been obtained, however, is mainly against this hypothesis: (i) It is not possible to dehydrate picrotoxinin to the corresponding diene, and it is conceivable that the molecular environment of the $C_4$ hydroxyl group of (I) might be such that a simple dehydration would be impossible. However, α-dihydropicrotoxinin (III on this formulation) should presumably undergo simple dehydration. It is, however, remarkably stable to acid dehydrating agents and was recovered unchanged after boiling with 85% formic acid, fused oxalic acid, or syrupy phosphoric acid. (ii) The oxidation of a structure such as (II; R = H) by the Oppenauer method, using acetone, would be expected to give (IV) (cf. Heilbron, Johnson, and Jones, *J.*, 1939, 1560) but *neo*picrotoxinin was recovered unchanged from such an experiment. (iii) The ozonolysis of *neo*picrotoxinin, formulated as (II; R = H), would be expected to give the ketone (V), and moreover, the same ketone should be obtained by ozonolysis of acetyl*neo*picrotoxinin (II; R = Ac, on this formulation). The study of this reaction is not yet completed, but the available evidence, particularly that bearing on the identity or otherwise of the two ozonolysis products, is against its taking this course.



(I.)



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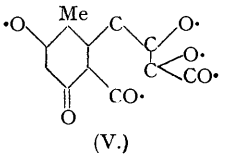
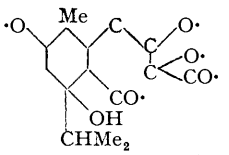
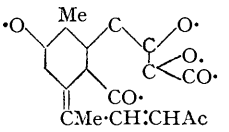


(V.)

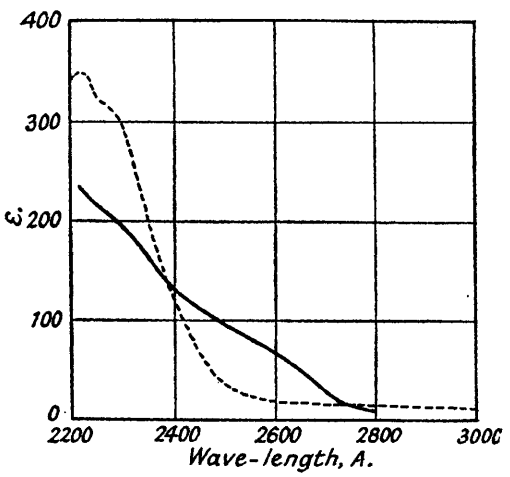
(III.)



(IV.)

Repeated ozonolysis of *neo*picrotoxinin, dissolved in ethyl acetate, gives in high yield a crystalline *substance*, $C_{12}H_{12}O_7$, m. p. 181—182°, whose molecular formula differs by the elements of water from that for (V). Unfortunately, this substance suffers some decomposition on prolonged heating even at moderate temperatures, and hence it was not possible to determine whether or not this loss is due to water of crystallisation, which is held tenaciously by many derivatives of picrotoxinin. The substance is neutral but devoid of carbonyl activity as shown by the non-formation of a 2 : 4-dinitrophenylhydrazone or semicarbazone, and the absence of any characteristic absorption in the ultra-violet absorption spectrum (see Fig.). It is a very strong reducing agent, reacting immediately in the cold with ammoniacal silver nitrate and alkaline potassium permanganate. This immediate reduction of alkaline permanganate appears to be a specific function of the newly generated functional grouping, since α-dihydropicrotoxinin and dihydro*neo*picrotoxinin do not show this reaction. With aqueous potassium hydroxide it reacts readily in the cold, giving a yellow solution turning red on warming and becoming colourless on acidification but without regeneration of the parent body. The same effect is observed if the substance is boiled with aqueous sodium hydrogen carbonate. It absorbs hydrogen under catalytic conditions (Adams's catalyst and acetic acid), although no pure substance has been isolated from the hydrogenation. The other product to be expected from the ozonolysis of (II; R = H) would be hydroxyacetone, and the behaviour of the




——— *Ozonolysis product of* neo*picrotoxinin.*
– – – – – „ „ „ *picrotoxinin.*

aqueous mother-liquor remaining from the decomposition of the ozonide of *neo*picrotoxinin was compatible with its presence. For instance, it gave a deep red colour with 40% aqueous potassium hydroxide, reduced ammoniacal silver nitrate in the cold immediately, and on distillation deposited a red resin and gave no detectable volatile carbonyl compound in the distillate. That the hydroxyl group has been lost on ozonolysis is indicated by the fact that the substance was recovered unchanged from an attempted acetylation (treatment with acetic anhydride and pyridine for two days at room temperature).

Ozonolysis of acetyl*neo*picrotoxinin also gives a second *substance*, $C_{12}H_{12}O_7$, m. p. 181—182°, but whereas the above compound from *neo*picrotoxinin is optically inactive, that obtained from acetyl*neo*picrotoxinin is optically active and the two show a mixed melting point depression. They also differ somewhat in their behaviour with aqueous potassium hydroxide, the compound from acetyl*neo*picrotoxinin showing little apparent reaction in the cold, and even on warming the crystals react only sluggishly, the solution becoming yellow.

It is evident from the published results (see especially Horrmann and Prillwitz, *Arch. Pharm.*, 1920, **258**, 200; Mercer and Robertson, *loc. cit.*; O'Donnell, Robertson, and Harland, *loc. cit.*) that the ozonolysis of picrotoxinin itself and its derivatives is by no means a simple reaction, and a further study of this is in progress. Preliminary experiments confirm the generation of formaldehyde, as described by Robertson and his co-workers, but the properties of the α-picrotoxinone formed at the same time, like those of the ozonolysis product of *neo*picrotoxinin, are somewhat anomalous. The analytical figures for the crystallised material which has been exhaustively dried agree with the required molecular formula $C_{14}H_{14}O_7$, but the ultra-violet absorption spectrum of the crude product (employed in preference to the crystallised material in order to eliminate the possibility of contamination with the β-modification; Horrmann and Prillwitz, *loc. cit.*) does not indicate the presence of a carbonyl group (see Fig.), and the characteristic semicarbazone, described by Horrmann and Prillwitz (*loc. cit.*), could not be prepared.

Like bromopicrotoxinin, bromo*neo*picrotoxinin reacts on warming with dilute sodium hydroxide solution, but while the former reaction is facile and irreversible by acidification, the latter is much slower and reversible, the corresponding free hydroxy-acid passing back very readily into the parent lactone. This appears to be the only example yet described of a member of the picrotoxinin series in which a lactone system can be reversibly opened. The crude bromo*neo*picrotoxinic acid can be readily obtained by precipitation of a cold aqueous solution of the sodium salt with mineral acid, but its crystallisation is difficult and on only one occasion was a satisfactory sample obtained; it then crystallised on standing in the ice-chest from a dilute acidified solution of the sodium salt. The analytical figures agree with $C_{15}H_{19}O_8Br$, which is regarded as the *hydrate* of *bromoneopicrotoxinic acid*, $C_{15}H_{17}O_7Br,H_2O$, the monocarboxylic acid corresponding to the α- and the β-bromopicrotoxinic acid, which retain water of crystallisation most tenaciously. The reversible nature of this reaction is considered to be of some importance. Its further study should be facilitated by stabilisation of the hydroxy-acid as the methyl ether. This methylation process has been little studied in the picrotoxin series, although Mercer and Robertson (*loc. cit.*) have described the conversion of dihydropicrotoxic acid into its dimethyl ether by the silver oxide method. There are obvious analogies between picrotoxinin, with its highly oxygenated side chain, and the sugars, and it seems likely that the investigation of its methylated derivatives will yield important results. The stability to alkali of the various brominated acids in this series suggested that the methyl sulphate method might be applicable, and a preliminary series of experiments has confirmed this in the case of β-bromopicrotoxinic acid, which can be converted into O-*methyl* β-*bromopicrotoxinic acid.*

## Experimental.

*Palladium Catalyst.*—Palladous chloride (0·2 g.), dissolved in concentrated hydrochloric acid (0·5 c.c.) and then diluted with water, was added to norit (2 g.) which had been heated to 140° in an evaporating dish with constant stirring, cooled in a vacuum desiccator, and mixed with water (100 c.c.). The catalyst mixture was reduced with hydrogen, and the palladium–charcoal catalyst then filtered off, washed with distilled water, absolute alcohol, and finally absolute ether, after which it was transferred to a vacuum desiccator.

*Hydrogenation and Bromination Experiments.*—(*a*) The following method gave reproducible yields of bromo*neo*picrotoxinin: Picrotoxin (2·0 g.), dissolved in alcohol, was shaken with the palladium–charcoal catalyst (0·4 g.) at atmospheric pressure in the presence of hydrogen. When the first relatively rapid absorption of hydrogen had ceased (usually about 35—40 c.c. during 15—120 minutes, depending on the activity of the particular sample of catalyst), the hydrogenation was interrupted, the catalyst filtered off, and the alcoholic solution evaporated in a vacuum on the water-bath. The residue, frequently obtained as a viscous gum, was dissolved by boiling with water (during this process any viscous material solidified to hard white granules which were ground to facilitate solution) and an excess of bromine water was then

added. After stirring and standing for a short while the reaction mixture deposited a white precipitate which was filtered off, washed with water, and dried; yield, 0·4 g. of clean but unpurified material. The delayed precipitation of bromo*neo*picrotoxinin is in sharp contrast to the immediate precipitation of bromopicrotoxinin under similar conditions. Crystallisation from alcohol (best by the use of a thimble placed in the hot vapours of a reflux condenser, as the solubility in alcohol is quite low) gave glistening needles of the compound previously described as "bromohydropicrotoxinin." The m. p. of this substance is dependent upon the conditions under which it is observed. The value previously quoted (254—255°, decomp., with previous shrinking) is obtained by heating in the usual way. If the bath is pre-heated to just a few degrees below the m. p., however, a much sharper and considerably higher m. p. can be observed, *viz.*, 265° (decomp.). In the sections which follow, m. p.s within this region have been obtained from a bath pre-heated to *ca.* 260°, and lower figures have been obtained from a bath pre-heated to 230—240°. The above product showed no depression in m. p. when mixed with a specimen of "bromohydropicrotoxinin."

(*b*) Picrotoxinin, treated as above, yielded the same bromo-derivative, m. p. (twice recrystallised) 262—263°.

(*c*) Palladous chloride (0·06 g.), dissolved in dilute hydrochloric acid (1 drop of concentrated hydrochloric acid + 4 c.c. of water), was added to a solution of picrotoxin (2·0 g.) in glacial acetic acid (25 c.c.). After hydrogenation for 50 minutes (uptake of hydrogen *ca.* 40 c.c.) the catalyst and solvent were removed and the residue was brominated; yield, 0·3 g. The product, crystallised from alcohol, had m. p. 253° (decomp.). There was no mixed m. p. depression with bromo*neo*picrotoxinin.

(*d*) When picrotoxinin in alcohol was shaken with the palladium–charcoal catalyst it was found, in agreement with the results of O'Donnell, Robertson, and Harland (*loc. cit.*), that no change occurred, the recovered material showing no mixed m. p. depression with the starting material.

(*e*) A specimen of a particularly active palladium–charcoal catalyst was shaken for several hours with aqueous alkali in the presence of hydrogen, filtered off, and washed repeatedly with hot water until the washings were only very slightly alkaline to phenolphthalein. By means of this neutralised catalyst, picrotoxinin (1·0 g.) was hydrogenated for 80 minutes (hydrogen uptake 32 c.c.), whereupon the experiment was stopped although slow absorption was still taking place. On bromination the product gave a bromo-derivative (0·6 g.), m. p. (after two crystallisations from alcohol) 268° (decomp.), which showed a strong mixed m.p. depression with bromo*neo*picrotoxinin but no depression with a sample of β-bromopicrotoxinin of approximately the same degree of purity.

(*f*) When a catalyst was used which had been made definitely alkaline to phenolphthalein, the absorption of hydrogen was negligible. After some time the hydrogenation was interrupted, a few drops of concentrated hydrochloric acid added, and shaking with hydrogen recommenced. Very little additional absorption took place, but the product on bromination yielded bromo*neo*picrotoxinin, m. p. 251° (decomp.) and showing no depression with authentic material.

(*g*) When picrotoxinin (1·0 g.) in alcohol was hydrogenated in the presence of Raney nickel catalyst, a very slow absorption took place (20 c.c. after 1½ hours' shaking and then standing overnight) but the product on bromination yielded only bromopicrotoxinin.

neo*Picrotoxinin*.—Bromo*neo*picrotoxinin (0·4 g.) in boiling alcohol (8 c.c.) was treated with a solution of ammonium chloride (0·08 g.) in water (0·4 c.c.), followed by zinc dust (0·12 g.) added in small quantities. Further additions of similar amounts of ammonium chloride solution and zinc dust were made, the volume of the reaction mixture being kept approximately constant by the addition of alcohol as required. After 20 minutes' boiling the product was clarified (norit) and filtered, giving a clear pale yellow solution which was evaporated to dryness. The residue was washed with a small quantity of 2N-sulphuric acid, then with cold water, and finally boiled with two successive portions of water to complete solution. Of the two crops of crystals obtained from these two extracts (0·05 g. and 0·06 g., respectively) the second was the cleaner and was further purified and examined. On recrystallisation it was obtained as fine white soft needles (0·04 g.), m. p. (bath pre-heated to 190°) *ca.* 204° after much softening, mixed m. p. with picrotoxinin, *ca.* 185° (Found, after drying in a vacuum desiccator overnight: C, 59·6; H, 6·0. $C_{15}H_{16}O_6,\frac{1}{2}H_2O$ requires C, 59·8; H, 5·65%). This neo*picrotoxinin hemihydrate* was again crystallised from water and then dried in a vacuum at 110° for several hours, giving the anhydrous neo*picrotoxinin*, m. p. *ca.* 212° (decomp.) after much previous softening (Found: C, 61·6; H, 5·5. $C_{15}H_{16}O_6$ requires C, 61·6; H, 5·5%). In subsequent preparations it was found that the yield varies with the period of heating of the bromo-derivative with the zinc, the highest yields (1·0—1·3 g. from 2·0 g. of bromo-derivative) being obtained by a short period. Under these conditions, also, the m. p. was somewhat higher (*ca.* 220°, decomp.) although still indefinite with much previous softening (Found: C, 61·4, 61·3; H, 5·5, 5·6%); $[\alpha]_D^{15.5°} - 18·8°$.

*Bromination of* neo*Picrotoxinin*.—When bromine water was added to a hot aqueous solution of *neo*picrotoxinin, a white precipitate formed on standing which on recrystallisation (twice) from alcohol melted at 254° (decomp.) and showed no depression with a specimen of bromo*neo*picrotoxinin, m. p. 256° (decomp.).

*Acetyl*neo*picrotoxinin*.—(*a*) *Indirect preparation from picrotoxinin* (O'Donnell, Robertson, and Harland, *loc. cit.*). Picrotoxinin (1·0 g.) was hydrogenated either in alcohol by use of a palladium–charcoal catalyst or in acetic acid with palladous chloride. A portion of the product was brominated, yielding bromo*neo*picrotoxinin, m. p. 257° (decomp.) undepressed by admixture with authentic material. The remainder (0·5 g.) was acetylated as described by Robertson *et al.* and crystallised from alcohol; yield, 0·2 g., m. p. 188°, raised by two further crystallisations to 190—190·5°.

(*b*) *Direct preparation from* neo*picrotoxinin*. *neo*Picrotoxinin (0·5 g.) was acetylated in the same way, giving a crude product (0·5 g.), m. p. *ca.* 180°, raised after three crystallisations from alcohol to 189—189·5° and showing no mixed m. p. depression with the above specimen (Found: C, 60·8; H, 5·5. Calc. for $C_{17}H_{18}O_7$: C, 61·1; H, 5·4%).

*Dihydro*neo*picrotoxinin*.—(*a*) *Indirect preparation from picrotoxinin* (Mercer and Robertson, *J.*, 1936, 288; O'Donnell, Robertson, and Harland, *loc. cit.*). Picrotoxinin (2·0 g.) in alcohol (100 c.c.) was hydrogenated in the presence of charcoal (1·0 g.) and palladous chloride (0·1 g.) in water (10 c.c.). After

6 hours the product was filtered, evaporated to small bulk, and diluted with water. The material which crystallised out on standing (1·1 g.) was heated under reflux for 12 hours with 1·5N-sulphuric acid (20 c.c.). After standing overnight, the "β-dihydropicrotoxinin" which was precipitated was crystallised from alcohol (charcoal); m. p. 255—256°.

(*b*) *Direct preparation from* neo*picrotoxinin.* *neo*Picrotoxinin (0·1 g.) in alcohol was hydrogenated overnight in the presence of an active palladium–charcoal catalyst, the uptake of hydrogen being *ca.* 100 c.c. The product, on crystallisation from aqueous alcohol, gave a small yield of *dihydro*neo*picrotoxinin*, m. p. 255—256° undepressed on admixture with the above "β-dihydropicrotoxinin" (Found: C, 61·1; H, 6·5. $C_{15}H_{18}O_6$ requires C, 61·2; H, 6·1%).

*Ozonolysis of* neo*Picrotoxinin.*—*neo*Picrotoxinin (1 g.) in absolute ethyl acetate (25 c.c.) was ozonised for 2 hours, the solvent distilled off in a vacuum at 30°, and the residual crystalline ozonide decomposed by standing overnight with water. The product was filtered off and crystallised from methanol and then ethanol; m. p. 181° (decomp.). The same product, m. p. and mixed m. p. 181°, was obtained when the period of ozonolysis was prolonged to 20 hours (Found: C, 54·5, 54·2; H, 4·7, 4·5%). This product gave a red colour in the cold with concentrated aqueous potassium hydroxide. When a sample of this once-ozonised *neo*picrotoxinin was dissolved in ethyl acetate and again ozonised for 2 hours, decomposed with water, and crystallised from ethanol, the resulting *product* had a slightly higher m. p. (182°, decomp., showing no depression when mixed with the starting material) and gave a yellow colour in the cold with concentrated aqueous potassium hydroxide, the red colour appearing only on heating. The analytical figures were also appreciably different (Found: C, 53·7, 53·9; H, 4·7, 4·5. $C_{12}H_{12}O_7$ requires C, 53·7; H, 4·5%).

*Ozonolysis of Acetyl*neo*picrotoxinin.*—Acetyl*neo*picrotoxinin (200 mg.) in ethyl acetate was ozonised and worked up as above; m. p. 181—182° (Found: C, 53·5; H, 4·8. $C_{12}H_{12}O_7$ requires C, 53·7; H, 4·5%); $[\alpha]_D^{12°}$ + 56°. A mixed m. p. depression was observed both with the starting material and with the above ozonolysis product of *neo*picrotoxinin. The aqueous mother-liquors remaining from the decomposition of the ozonide reduced ammoniacal silver nitrate only an heating, and with concentrated aqueous potassium hydroxide gave no colour in the cold but developed a yellow colour on heating.

*Ozonolysis of Picrotoxinin.*—Picrotoxinin (5 g.) in ethyl acetate (200 c.c.) was ozonised until a specimen of the reaction mixture after evaporation and solution in boiling water no longer gave a precipitate with bromine water (*ca.* 18 hours). After the solvent had been removed in a vacuum at 30° the residual solid ozonide was treated with water, broken up with a stirring rod, and set aside overnight. It was then filtered off, and a specimen crystallised from aqueous alcohol. Without special drying the crystals thus obtained shrank immediately when placed in a melting-point bath at 110°. The m. p. was quite indefinite. The product was therefore dried for a prolonged period in a vacuum over phosphoric oxide, first at room temperature and finally at a temperature which was gradually increased to 90°. The m. p., from a bath preheated to 160°, was now *ca.* 180° after much previous softening (Found: C, 56·7; H, 4·6. Calc. for $C_{14}H_{14}O_7$: C, 57·2; H, 4·8%). The aqueous mother-liquors remaining from the decomposition of the ozonide were distilled to small bulk and the distillate treated with an excess of aqueous 2 : 4-dinitrophenylhydrazine hydrochloride. The resulting yellow precipitate was collected and crystallised from alcohol; m. p. 163° undepressed with authentic formaldehyde 2 : 4-dinitrophenylhydrazone.

*Reaction of Bromo*neo*picrotoxinin with Alkali.*—Bromo*neo*picrotoxinin (0·1 g.) was warmed to solution with 2N-sodium hydroxide and then acidified with dilute mineral acid. On standing in the ice-chest, *bromo*neo*picrotoxinic acid* crystallised out in beautiful needles (0·06 g.), m. p. 225—226° (decomp.) from a bath preheated to 220° (Found: C, 44·3; H, 4·9; Br, 18·7,* 21·5.* $C_{15}H_{19}O_8Br$ requires C, 44·2; H, 4·7; Br, 19·7%). In a further preparation, bromo*neo*picrotoxinin (0·5 g.) was dissolved by boiling with dilute aqueous sodium hydroxide, and the cold solution was acidified with a little concentrated hydrochloric acid. The crude precipitated material (0·42 g.) was taken up in sodium hydrogen carbonate solution and reprecipitated with acid. The m. p., taken in the usual way, was *ca.* 217° after very noticeable shrinking and blackening at lower temperatures, but when placed in a pre-heated bath it melted immediately. By trial, the true m. p. (clean decomposition without blackening) appears to be 195—196° from a bath pre-heated to 193°. In one of several attempts to recrystallise the acid, it was dissolved in chloroform, some light petroleum (b. p. 60—80°) added, and the resulting slightly cloudy solution dried ($Na_2SO_4$) and filtered. On slow evaporation at room temperature well-formed crystals were left, but satisfactory analytical figures were not obtained, much solvent of crystallisation being retained. When the crude acid was crystallised from dilute acetic acid, well-formed crystals were obtained, m. p. 245° (decomp.) unchanged by recrystallisation from glacial acetic acid and showing no mixed m. p. depression with bromo*neo*picrotoxinin (Found: C, 48·2; H, 4·2. Calc. for $C_{15}H_{15}O_6Br$: C, 48·5; H, 4·0%). The re-formation of the lactone from the acid was frequently observed in the course of its examination.

O-*Methyl β-Bromopicrotoxinic Acid.*—β-Bromopicrotoxinic acid (0·5 g.) was treated dropwise with methyl sulphate (0·5 g.) and 40% aqueous potassium hydroxide so that the reaction mixture was always slightly alkaline. On acidification, the hot solution rapidly precipitated an *acid*, which was filtered off, dried (yield, 0·3 g.), and crystallised from water and then alcohol; m. p. 244° (decomp.) (Found: C, 46·8; H, 4·5; Br, 20·3 *; OMe, 7·7.* $C_{16}H_{19}O_7Br$ requires C, 47·7; H, 4·7; Br, 19·8; OMe, 7·7%). A strong mixed m. p. depression was observed with the starting material.

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UNIVERSITY OF OTAGO, DUNEDIN, NEW ZEALAND.
CHEMICAL LABORATORIES, CAMBRIDGE.

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* Analyses by Drs. Weiler and Strauss, Oxford.