267. *Picrotoxin. Part IV.*

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An extensive investigation on the hydrogenation of picrotoxinin under a variety of conditions was undertaken and, whilst only α-dihydropicrotoxinin was obtained when a platinum catalyst was employed, the use of a palladium catalyst invariably gave a mixed product, the constituents of which appear to depend on the solvent.

As tested by hydrogenation and ozonolysis methods, α- and β-bromopicrotoxinin, the corresponding α- and β-bromopicrotoxinic acids, and β-picrotoxinic acid do not contain a double bond. On the other hand α-picrotoxinic acid has an ethylenic linkage present in an *iso*propylene system, since on ozonolysis it yields a product which on being boiled with hydriodic acid and red phosphorus gives rise to the ketone $C_{13}H_{16}O_2$ and nor- and hydroxynor-picrotic acid.

IN Part II (J., 1936, 288) it was recorded that on hydrogenation with a palladium catalyst in aqueous alcohol containing small amounts of hydrochloric acid picrotoxinin gave rise to a mixed product which had m. p. varying from 227° to 234° according to the experiment and could be partly converted into picrotonol under comparatively mild conditions. Since the formation of an aromatic from the hydroaromatic system of picrotoxinin and its derivatives appears to be of considerable value in the elucidation of constitution in this series a more detailed inquiry into the formation of the picrotonol precursor seemed highly desirable and we therefore initiated an extensive investigation on the hydrogenation of picrotoxinin with a palladium catalyst under a wide variety of conditions. Of the mass of data which has been collected, only the results of typical experiments are recorded in the experimental section; the use of a platinum catalyst under the same conditions invariably gave only α-dihydropicrotoxinin (Part II, *loc. cit.*).

In the course of attempts to prepare a quantity of β-dihydropicrotoxinin according to the method employed by Mercer and one of us (A. R.) (Part II, *loc. cit.*) we have, in spite of very many experiments under a variety of conditions, failed so far to repeat this preparation. Instead, the hydrogenation proceeded more slowly and we invariably obtained a neutral product which could not be resolved into pure constituents by means of solvents, but on being boiled with 5% sulphuric acid gave rise to a mixture from which picrotonol, β-dihydropicrotoxinin, and dihydropicrotoxic acid were isolated. In view of the fact that under these conditions β-dihydropicrotoxinin is unaffected by boiling acid and α-dihydropicrotoxinin is quantitatively converted into dihydropicrotoxic acid it would seem that in all probability the hydrogenation product contained both α- and β-dihydropicrotoxinin. Further, since neither of the latter compounds yields picrotonol on being boiled with aqueous sulphuric acid, the hydrogenation mixture also contained the ketol precursor. We are at present unable to offer a satisfactory explanation of the failure to obtain a quantitative yield of β-dihydropicrotoxinin, but it may well be that this hydrogenation, which in the earlier experiments proceeded rapidly, requires an exceptionally active palladium catalyst and that our specimens of picrotoxinin may have contained minute traces of metallic

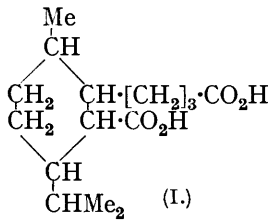
" poisons " carried down from the debromination process employed in its preparation (Horrmann's method, *Arch. Pharm.*, 1920, **258**, 200).

Although so far the isolation of the picrotonol precursor has not been achieved, it has been found that the hydrogenation of picrotoxinin, dissolved in acetic acid containing small amounts of hydrochloric acid, yields a neutral product which appears to be a eutectic mixture consisting of α-dihydropicrotoxinin and the required compound only. This conclusion follows from the fact that on hydrolysis with acid the hydrogenation product gave rise to a mixture of picrotonol and dihydropicrotoxic acid which did not contain detectable amounts of β-dihydropicrotoxinin. Unlike the β-compound, α-dihydropicrotoxinin does not form an acetate (Part II, *loc. cit.*) and accordingly by acetylation of the hydrogenation product it was found possible to isolate a small amount of the *acetate* of the picrotonol precursor, which on being boiled with dilute sulphuric acid gave an almost quantitative yield of ketol. That this compound is the acetate of an isomeride of picrotoxinin, *i.e.*, $C_{15}H_{15}O_6(CO{\cdot}CH_3)$, and not of a dihydropicrotoxinin appears likely for the following reason: Picrotonol, which so far has not been obtained by direct hydration of picrotoxinin, was originally prepared by the action of boiling 25% sulphuric acid on picrotin (Angelico, *Gazzetta*, 1910, **40**, i, 391). The latter substance closely resembles picrotoxinin, *e.g.*, in exhibiting reducing properties and in forming α-picrotinic acid and picrotindicarboxylic acid, and may be considered to be a hydrate of picrotoxinin formed by addition of the elements of water at the double bond, a view which finds support in the fact that, on being heated, α-picrotinic acid yields picrotoxic acid in addition to picrotin lactone (Horrmann, *Annalen*, 1916, **411**, 273). Thus for picrotonol formation from the precursor under the conditions employed, a compound having the same state of oxidation as picrotin is in all probability an intermediate stage and this could arise by direct hydration of a suitable isomeride of picrotoxinin but not of a dihydropicrotoxinin. The mechanism of the formation of the picrotonol precursor is at present obscure, but it seems probable that hydrogen plays an essential part, because numerous attempts to effect isomerisation of picrotoxinin by active catalysts in the absence of hydrogen failed completely. In this connection it may be noted that, whilst both α- and β-dihydropicrotoxinin retain the reducing properties of picrotoxinin, only the α-compound can be converted into a monobasic acid, dihydropicrotoxic acid, on being boiled with dilute mineral acids, and therefore in all probability this derivative is formed by the direct addition of hydrogen at the double bond of picrotoxinin. On the other hand the formation of the β-compound appears to be accompanied by an isomeric change, because it cannot be converted into an acid under the conditions employed for the hydration of α-dihydropicrotoxinin and, further, whereas the hydroxyl group in picrotoxinin and α-dihydropicrotoxinin, detected by the method of Zerewitinoff cannot be acetylated, β-dihydropicrotoxinin readily forms an *acetate*, thus indicating that the production of the β-derivative is probably accompanied by suppression of the tertiary hydroxyl and the formation of a secondary hydroxyl group (a primary alcohol group is regarded as exceedingly unlikely).

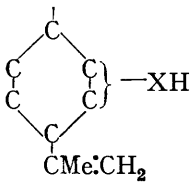
When alcohol containing small amounts of hydrochloric acid was employed as the medium for the hydrogenation of picrotoxinin with a palladium catalyst, a product was obtained which could be resolved into a neutral and an acidic fraction. From the neutral portion it has been possible by a tedious fractional crystallisation to isolate α- and β-dihydropicrotoxinin and to show that this fraction also contained the picrotonol precursor. The acidic fraction appeared to consist mainly of a saturated *acid*, $C_{15}H_{22}O_6$, which on titration was found to be dibasic and on treatment with diazomethane gave a *dimethyl* ester. Since it does not react with 2 : 4-dinitrophenylhydrazine, the acid does not appear to contain a carbonyl group and the two remaining oxygen atoms are probably present as hydroxyl groups. From the established *C*-skeleton of picrotoxinin, which depends on the structure of picrotone, picrotonol and picrotic acid, it is tentatively suggested that the acid $C_{15}H_{22}O_6$ may be a dihydroxy-derivative of (I).

In view of the foregoing results obtained with picrotoxinin the behaviour of a number of its derivatives towards hydrogen in the presence of an active catalyst has been examined. It has been found that α- and β-bromopicrotoxinin and the respective α- and β-bromopicrotoxinic acids, which are formed by the action of 10% potassium hydroxide solution

on these compounds (Horrmann, *Ber.*, 1913, **46**, 2796), cannot be hydrogenated. The absence of an ethylenic linkage in these substances is confirmed by the fact that they are unaffected by prolonged treatment with a mixture of ozone and oxygen. On the other hand α-picrotoxinic acid, which is formed by debromination of α- or β-bromopicrotoxinic acid in a manner analogous to the formation of picrotoxinin from its bromo-derivatives, can be readily hydrogenated, yielding a dihydro-derivative, and on ozonolysis gives rise to formaldehyde and a ketonic compound. On being boiled with hydriodic acid containing red phosphorus, the latter substance, which has not so far been obtained crystalline and may be a mixture of two forms corresponding to α- and β-picrotoxinone, furnished a mixture from which products identical with those obtained from picrotoxinone have been isolated, *viz.*, the phenolic ketone $C_{13}H_{16}O_2$, nor-, and hydroxynor-picrotic acid, a result which clearly shows the ethylenic linkage of α-picrotoxinic acid is in the same position as in picrotoxinin and picrotoxic acid, *i.e.*, forming an *iso*propylene system. Curiously enough, β-picrotoxinic acid, which is formed by isomerisation of the α-compound, does not appear to contain a double bond, since it cannot be hydrogenated or oxidised with ozone.



(I.)



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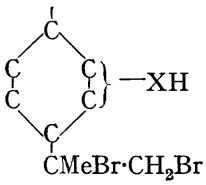

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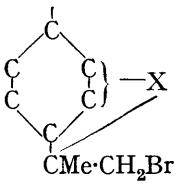

(IV.)

Although decisive experimental evidence regarding the mechanism of the formation of the saturated α- and β-bromopicrotoxinin from picrotoxinin or of α- and β-bromopicrotoxinic acid from the unsaturated α-picrotoxinic acid is lacking, it seems likely that the bromination reaction takes the same course in each case and the following explanations are tentatively advanced: The addition of bromine to the unsaturated system (II) may be considered to take the normal course, giving rise to the intermediate dibromo-derivative type (III), but this reaction is accompanied by loss of hydrogen bromide so as to form a new cyclic system type (IV) (where X may be a C or an O atom); the pairs of α- and β-forms are considered to be stereoisomers. In the debromination process the replacement of the bromine atom in (IV) with hydrogen is accompanied by scission of the cyclic system, thus regenerating (II). Alternatively, since the reaction is carried out in aqueous solution, the formation of the saturated bromo-compounds may take place by the addition of the elements of hypobromous acid at the double bond, giving type (III, where one of the bromine atoms is replaced by a hydroxyl group), with subsequent loss of water from the latter resulting in the formation of (IV, X = O).

In the course of attempts to obtain picrotoxinin dibromide it was found that treatment of anyhydrous picrotoxinin with bromine in chloroform gave a highly unstable product, which partly decomposed with the evolution of hydrogen bromide during isolation and was not obtained crystalline; smaller amounts of hydrogen bromide were also given off during the bromination process. The crude material, on being warmed with water, gave in quantitative yield a mixture of α- and β-bromopicrotoxinin and hydrobromic acid, a result which appears to support the first explanation of the bromination mechanism.

Experimental.

Hydrogenation of Picrotoxinin.—(A) *With a palladium–charcoal catalyst in ethyl acetate.* When a solution of picrotoxinin (5 g.) in absolute ethyl acetate, containing a palladium–charcoal catalyst (from 0·3 g. of palladium chloride and 2 g. of charcoal), was agitated in an atmosphere of hydrogen, absorption ceased in 30—50 minutes (vol. absorbed, 120—160 c.c.; theoretical, 383 c.c.). From the crystalline non-acidic residue, m. p. between 210° and 225° according to experiment, which was left on evaporation of the filtered solution, a pure product could not be isolated by repeated crystallisation from a variety of solvents. The crude product (0·8 g.) was boiled with N-sulphuric acid for 23 hours and on being cooled the resulting solution deposited

β-dihydropicrotoxinin (0·1 g.), which formed elongated prisms, m. p. 254—255°, from ethyl acetate, undepressed by admixture with an authentic specimen (Part II, *loc. cit.*). Acetylation of this compound (1 g.) with acetic anhydride (4 c.c.) and pyridine (2 c.c.) at room temperature during 10 days gave the *acetate*, which separated from ethyl alcohol in colourless prisms, m. p. 177° (Found: C, 60·7; H, 6·2. $C_{17}H_{20}O_7$ requires C, 60·7; H, 6·0%).

From the acidic filtrate which was left on separation of the crude β-dihydropicrotoxinin and which had been neutralised with sodium bicarbonate, picrotonol was isolated as a colourless viscous oil by means of ether and converted into the semicarbazone (0·2 g.), which on purification had m. p. 222°, undepressed by admixture with an authentic specimen (Part II, *loc. cit.*). The neutral aqueous liquors were then acidified with hydrochloric acid and thoroughly extracted with ether, and the combined dried extracts evaporated, leaving dihydropicrotoxic acid, m. p. and mixed m. p. 254°, after purification from ethyl acetate.

(B) *With a palladium catalyst in alcohol.* When a solution of palladium chloride (0·2—0·3 g.) in water (20—50 c.c.) containing 2 g. of charcoal was added to a solution of picrotoxinin (6 g.) in absolute alcohol (250—350 c.c.), and the mixture agitated in hydrogen at atmospheric pressure, the absorption of gas, which was rapid at first and then gradually decreased, was complete in 3—6 hours; the volume absorbed (910—1050 c.c.) was approximately equivalent to 2 mols. On being boiled with N-sulphuric acid for 19 hours, the crystalline residue, m. p. 90—120° according to experiment, left on evaporation of the filtered solution in a vacuum gave rise to β-dihydropicrotoxinin, picrotonol, and dihydropicrotoxic acid, which were isolated from the resulting mixture by the method employed in (A). If, instead of being evaporated to dryness, the volume of the filtered hydrogenation solution was reduced to about 50 c.c. and the residue cooled, β-dihydropicrotoxinin (1·2—1·9 g.), m. p. between 248° and 254°, separated and on recrystallisation from ethyl acetate had m. p. 256°, alone or mixed with an authentic specimen. The product (3 g.), m. p. about 225°, obtained by addition of water to the concentrated reaction mixture also consisted mainly of β-dihydropicrotoxinin. In either case the filtrate from the crude β-dihydropicrotoxinin contained the acidic fraction (X) and it was ultimately observed that the crude hydrogenation product could be conveniently resolved into a neutral and an acidic fraction by means of aqueous sodium bicarbonate.

On being boiled with N-sulphuric acid, the neutral fraction (2 g. of a sample, m. p. 208—209°) yielded β-dihydropicrotoxinin, dihydropicrotoxic acid, and picrotonol [the last being characterised by conversion into the *p*-nitrobenzoate, m. p. and mixed m. p. 155° (Part II, *loc. cit.*) after crystallisation from alcohol] and consequently this fraction was subjected to a prolonged fractional crystallisation. On being cooled, a solution of the neutral material (7·8 g. from several experiments) in hot ethyl acetate deposited β-dihydropicrotoxinin (0·4 g.), m. p. 254°, and on addition of an excess of light petroleum (b. p. 60—80°) to the ethyl acetate liquors a solid (7·2 g.), m. p. 215—217°, separated. Crystallised from benzene (1 l.), the latter material gave a solid (3·6 g.), m. p. 217—218°, which was resolved by means of alcohol into a less soluble fraction and a soluble residue (2·3 g.), m. p. 217—218°. From the former fraction β-dihydropicrotoxinin was obtained by repeated crystallisation from alcohol; the residue, m. p. 217—218°, on being recrystallised once from water, three times from alcohol, and finally from acetic acid, gave a small amount of a product, m. p. 219—220° (Found: C, 61·2; H, 6·4. Calc. for $C_{15}H_{18}O_6$: C, 61·2; H, 6·1%). On admixture with α-dihydropicrotoxinin this material melted at 222—223° and with β-dihydropicrotoxinin at 219—220°.

Concentration of the benzene filtrate gave a solid (1·6 g.), m. p. 227—228°, which on being crystallised four times from alcohol and then repeatedly from acetic acid yielded a small amount of α-dihydropicrotoxinin.

The initial acidic fraction, which varied in amount between 25 and 50% of the picrotoxinin used and was obtained as either a very viscous oil or a crystalline solid, was apparently identical with the fraction (X) and on being boiled with 2N-sulphuric acid the crude material yielded a small amount of a neutral, pale yellow oil which did not contain picrotonol. Crystallised from water (resulting in the loss of much material; other solvents proved unsuitable), it gave an *acid* in slender needles, m. p. 183° (efferv.), which appeared to be a hydrate (Found in material dried over phosphoric oxide at room temperature: C, 57·9; H, 7·7; H_2O, 5·7. $C_{15}H_{22}O_6,H_2O$ requires C, 57·0; H, 7·6; H_2O, 5·7%. Found in a specimen dried in a vacuum at 100°: C, 60·5; H, 7·6. $C_{15}H_{22}O_6$ requires C, 60·4; H, 7·4%). Esterification of this compound with an excess of ethereal diazomethane gave a neutral *ester*, which separated from light petroleum in slender needles, m. p. 164° [Found: C, 62·6; H, 8·0; OMe, 18·8. $C_{15}H_{20}O_4(OMe)_2$ requires C, 62·6; H, 8·0; OMe, 19·0%].

When the hydrogenation of picrotoxinin (6 g.) was carried out with a palladium chloride

catalyst in alcohol in the absence of a charcoal carrier, the absorption of hydrogen (460—510 c.c.; a slight excess over that required to saturate one double bond) ceased after 4—5 hours and the product contained a somewhat larger proportion of neutral material (3·8—4·4 g.) and a correspondingly smaller acidic fraction. The former was shown to contain α- and β-dihydropicrotoxinin and the picrotonol precursor; the latter consisted largely of the acid, m. p. 183°.

(C) *With a palladium catalyst in acetic acid.* Palladium chloride (0·3 g.), dissolved in water (20 c.c.) containing 5 drops of hydrochloric acid, was added to a solution of picrotoxinin (5 g.) in absolute acetic acid (125 c.c.), and the mixture agitated in an atmosphere of hydrogen. Absorption (160—180 c.c., equivalent to 0·4—0·6 mol.) ceased in 4—6 hours and on evaporation the filtered solution left a crystalline product, m. p. between 220° and 230°, which did not contain acidic material and on repeated crystallisation from water, benzene, and finally alcohol gave an apparently homogeneous substance in colourless prisms, m. p. 232° (Found: C, 61·4; H, 6·0. Calc. for $C_{15}H_{16}O_6$: C, 61·6; H, 5·5. Calc. for $C_{15}H_{18}O_6$: C, 61·2; H, 6·1%), but on being boiled with 5% sulphuric acid for 20 hours this material (1·8 g.) gave rise to picrotonol (0·5—0·6 g., identified as semicarbazone) and dihydropicrotoxic acid (0·9 g.).

Addition of ice-water to a solution of the crude reaction product (0·5 g., m. p. 228—230°) in acetic anhydride (3 c.c.) and pyridine (1·5 c.c.), which had been kept at room temperature for 10 days, precipitated a solid (0·5 g.), m. p. between 125° and 160°, which on being once recrystallised from alcohol had m. p. 187°; yield 0·25 g. Repeated purification from the same solvent finally gave the *acetate* in well-formed rod-like prisms, m. p. 190°, which on admixture with the acetate of β-dihydropicrotoxinin, m. p. 177°, melted at about 166° [Found: C, 61·2; H, 5·5; CH_3·CO, 15·5. $C_{15}H_{15}O_6(CH_3{\cdot}CO)$ requires C, 61·1; H, 5·4; CH_3·CO, 12·9%]. This compound (1 g.) was boiled with N-sulphuric acid (40 c.c.) for 20 hours, and the cooled solution neutralised with sodium bicarbonate and repeatedly extracted with ether. Evaporation of the combined dried extracts left picrotonol (0·4 g.), which gave a quantitative yield of the semicarbazone. Extraction of the acidified aqueous liquors with ether gave a small amount of a glassy solid (< 0·1 g.) which could not be purified.

Dihydro-α-picrotoxinic Acid.—Absorption of hydrogen (approx. 1 mol.) by α-picrotoxinic acid (Horrmann, *Ber.*, **1913**, **46**, 2796) (2 g.), dissolved in acetic acid (125 c.c.) containing a platinum oxide catalyst (0·1 g.), was complete in less than 5 minutes. Removal of the catalyst and then the solvent left the dihydro-acid (2 g.), m. p. 229°, which separated from water in colourless diamond-shaped plates, m. p. 231° (Found: C, 57·4; H, 6·4. Calc. for $C_{15}H_{20}O_7$: C, 57·7; H, 6·5%) (compare Horrmann, *loc. cit.*). This acid, which is soluble in alcohol or acetone and sparingly soluble in ethyl acetate or benzene, does not reduce Fehling's solution and does not decolorise bromine water.

Application of the same procedure to β-picrotoxinic acid gave unchanged material.

Ozonolysis of α-Picrotoxinic Acid.—A stream of ozone and oxygen was passed into a solution of the acid (1 g.) in absolute ethyl acetate (60 c.c.) for 5 hours, the solvent was evaporated, and the residue hydrolysed with water at room temperature for 24 hours and then on the water-bath for 15 minutes. Evaporation of the clear liquid in a vacuum over sulphuric acid slowly at room temperature or in a vacuum on the steam-bath left an acid as a hygroscopic glassy solid, from which crystalline material was not obtained. This product, which was readily soluble in water, alcohol or acetone and sparingly soluble in benzene, readily formed an amorphous 2 : 4-dinitrophenylhydrazone which could not be purified. In another experiment the aqueous hydrolysate was distilled with the continuous addition of water to keep the volume constant and on treatment with an excess of a solution of 2 : 4-dinitrophenylhydrazine hydrochloride the distillate (70 c.c.) gave a crystalline precipitate of formaldehyde-2 : 4-dinitrophenylhydrazone which had m. p. 164° after purification and was identified by comparison with an authentic specimen; yield, approx. 35% of the theoretical.

When a mixture of the glassy solid (9·2 g., from 10 g. of α-picrotoxinic acid), hydriodic acid (25 c.c.; *d* 1·7), and red phosphorus (4 g.) was gently warmed, a vigorous reaction ensued; after this inital reaction had ceased, the dark liquid was refluxed for 6 hours, cooled, and diluted with water (400 c.c.). Next day the clear solution was decanted from the viscous tarry residue, saturated with sodium chloride, and extracted with ether. The concentrated ethereal extract (250 c.c.) was decolorised with sulphurous acid, washed with water, and extracted with aqueous sodium bicarbonate (12 × 10 c.c.). Acidification of the combined extracts precipitated crude hydroxynorpicrotic acid (0·9 g.), which was repeatedly crystallised from benzene–acetone and then from aqueous acetone, forming glistening plates, m. p. 213°, undepressed on admixture with an authentic specimen (Found in a specimen dried in a vacuum at 100°: C, 63·3; H, 6·1. Calc. for $C_{14}H_{16}O_5$: C, 63·6; H, 6·1%).

An ethereal extract (250 c.c.) of the viscous residue was decolorised with sulphurous acid and extracted with aqueous sodium bicarbonate (18 × 10 c.c.). The dark-coloured acidic product (3·2 g.) obtained by acidification of the combined bicarbonate extracts was esterfied with ethyl alcohol, and the ester purified by repeated distillation in a vacuum, being obtained as an almost colourless oil (0·8 g.), b. p. 140—160°/1 mm., which was hydrolysed with boiling 8% aqueous-alcoholic sodium hydroxide for 12 hours. The resulting crude norpicrotic acid was repeatedly purified from warm water and then aqueous alcohol, forming slender prisms, m. p. 113°, identical in every way with an authentic specimen (Found in material dried in a vacuum at 80° : C, 67·5; H, 6·6. Calc. for $C_{14}H_{16}O_4$: C, 67·7; H, 6·5%).

The combined ethereal solutions left on separation of nor- and hydroxynor-picrotic acid were extracted with 1% aqueous sodium hydroxide (10 × 10 c.c.) and on acidification the extracts deposited the phenolic ketone (0·2 g.), which had m. p. and mixed m. p. 189°, after recrystallisation from carbon tetrachloride, followed by sublimation in a high vacuum. The 2 : 4-dinitrophenylhydrazone separated from ethyl acetate in slender red needles, m. p. 286°, undepressed by admixture with an authentic specimen. Evaporation of the residual ethereal solution left a dark viscous neutral oil (0·9 g.) which could not be purified.

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