293. *Picrotoxin and Tutin. Part V.* The Dehydration of Picrotin and Some Alkaline Degradations.*

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(With an Appendix by M. Sutter and E. Schlittler.)

Dehydration of picrotin with oxalic acid gives a mixture which on bromination yields bromo*neo*picrotoxinin and a second bromo-derivative not identical with any known bromopicrotoxinin. Degradation of dihydro-*neo*picrotoxinin with hot sodium carbonate solution gives δ-hydroxy-γ-keto-β-*iso*propylvaleric (I) and 5-hydroxy-3-keto-2-methyl*cyclo*pent-1-ene-1-carboxylic acid (II), whereas picrotin under the same conditions yields the *cyclo*pentene acid and an acid which may be δ-hydroxy-β-(1-hydroxy-1-methylethyl)-γ-ketovaleric acid (III). A convenient method of separating (I) and (II) is described. An appendix gives details of the oxidation of dihydropicrotoxinin to *iso*butyric acid and of picrotin to α-hydroxy*iso*butyric acid.

In Parts III and IV (*J.*, 1949, 806; 1952, 185) we discussed the functional groups of picrotin and picrotoxinin. We now record experiments designed to elucidate the relation between the two substances. It has long been suspected that this must be close, for, *e.g.*, each can be converted into picrotic acid (Angelico, *Gazzetta*, 1911, **41**, ii, 337), the chloro-ketone $C_{14}H_{15}O_3Cl$ (*idem*, *Atti R. Accad. Lincei*, 1910, [v], **19**, i, 473), and picrotonol (*idem*, *Gazzetta*, 1910, **40**, i, 391; O'Donnell, Robertson, and Harland, *J.*, 1939, 1261;

* Part IV, *J.*, 1952, 185.

Slater, *J.*, 1949, 806). The simplest assumption would be that picrotin, $C_{15}H_{18}O_7$, is related to the olefin picrotoxinin, $C_{15}H_{16}O_6$, by simple addition of water :

Picrotoxinin, $C_{12}H_{11}O_6 \cdot C(:CH_2) \cdot CH_3$ Picrotin, $C_{12}H_{11}O_6 \cdot C(CH_3)_2 \cdot OH$

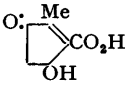
and Schlittler and Sutter (Sect. 6, 1st Int. Congr. Biochemistry, Cambridge, 1949) reported that, in agreement with this, oxidation of dihydropicrotoxinin with alkaline permanganate yields *iso*butyric acid whereas picrotin yields α-hydroxy*iso*butyric acid. Nevertheless no direct interconversion has yet been recorded, the nearest approach being the dehydration of α-picrotinic acid by heat to picrotoxic acid (Horrmann, *Annalen*, 1916, **411**, 273) :

$$\begin{array}{ccc} \text{Picrotin, } C_{15}H_{18}O_7 & \xrightarrow[\text{acid}]{\text{Mineral}} & \alpha\text{-Picrotinic acid, } C_{15}H_{20}O_8 \\ & & \downarrow -H_2O \\ \text{Picrotoxinin, } C_{15}H_{16}O_6 & \xrightarrow[\text{acid}]{\text{Mineral}} & \text{Picrotoxic acid, } C_{15}H_{18}O_7 \end{array}$$

However, since both picrotin and dihydropicrotoxinin are complex substances readily degraded by alkali, the products of oxidation under alkaline conditions can give little information about the molecule as a whole (the Swiss authors apparently recognise the existence of as yet undefined differences between picrotin and picrotoxinin). In any case, until the relations of α-picrotinic acid to picrotin, and of picrotoxic acid to picrotoxinin, are determined, there is no direct evidence for the above simple olefin–hydrate relation.

A further link between the two series has now been found in the degradation of picrotin to the acid (II) previously isolated from the products of alkaline degradation of picrotoxinin, dihydropicrotoxinin, α-picrotoxinic acid, and dihydro-α-picrotoxinic acid (Sutter and Schlittler, *Helv. Chim. Acta*, 1949, **32**, 1855, 1864; 1950, **33**, 902). When a picrotin solution containing sodium hydrogen carbonate is concentrated by boiling, it rapidly becomes brown and no picrotin is recovered. When heated with carbonate solution, picrotoxinin behaves similarly. This suggested that fission of the picrotin molecule might be taking place and further investigation confirmed this. With the hope that this method might be valuable if applied to tutin we first adapted Sutter and Schlittler's methods

$HO \cdot CH_2 \cdot CO \cdot \underset{\displaystyle CHMe_2}{\underset{|}{CH}} \cdot CH_2 \cdot CO_2H$ (I)



(II)

$HO \cdot CH_2 \cdot CO \cdot \underset{\displaystyle HO \cdot CMe_2}{\underset{|}{CH}} \cdot CH_2 \cdot CO_2H$ (III)

to the working-up of small quantities of degradation products. Since (II) is a stronger acid than (I), the accompanying degradation product of dihydropicrotoxinin, and the carbonate degradation solution acts as a buffer, acidification to pH *ca.* 6 leaves (II) almost completely as its anion while (I) is almost completely un-ionised and is readily removed by continuous extraction with ether. Further acidification and ether-extraction of the solution then removes (II). The constants of the two acids agreed with those recorded by Sutter and Schlittler, except that in the spectrum of (I) we found $\lambda_{max.}$ 279 mμ whereas the Swiss workers give 260 mμ. Professor Schlittler has since advised us that the latter figure should have read $\lambda_{max.}$ 276 mμ. Alternatively (I) and (II) were separated by using an ion-exchange column.

The alkaline degradation of picrotin was then attempted and yielded finally (II) and a more weakly acidic fraction which proved extremely difficult to manipulate, largely owing to its tendency to revert to an oily lactone. Neither the free acid nor the lactone could be obtained pure. The properties, and the lack of carbonyl activity, are very similar to those of (I) and are not incompatible with the structure (III) which is that of the product of alkaline degradation to be expected if the carbon skeletons of picrotin and picrotoxinin are identical. Further, we have found that dihydro*neo*picrotoxinin is degraded in hot carbonate solution to (I) and (II), and this taken in conjunction with the dehydration of picrotin to *neo*picrotoxinin (see below) and the evidence cited at the beginning of this paper provides support for this view.

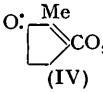
In the light of the above results the failure of previous workers to dehydrate picrotin

to a " picrotoxinin " appeared to us most unexpected unless that hydroxyl group which appears in α-hydroxybutyric acid and (III)—both isolated from *alkaline* reaction mixtures—is bound by lactone formation in picrotin itself. We therefore tried to dehydrate the sodium salt of the " true picrotinic acid " (the as yet unisolated hydroxy-acid formed as the salt when picrotin is dissolved in cold, dilute, aqueous sodium hydroxide). When the dry sodium salt was fused with oxalic acid and the product brominated, a small amount of bromo*neo*picrotoxinin was isolated (Slater and Wilson, *Nature*, **1951**, **167**, 324). When picrotin itself was fused with oxalic acid and brominated, it yielded, somewhat unexpectedly, a mixture of bromo*neo*picrotoxinin and another bromo-derivative; yields were very small and variable and it has not been possible to obtain sufficient of this new material for a full investigation. It is similar to the known bromopicrotoxinins (α-, β-, and *neo*-) in giving with alkali an acid somewhat similar to α- and β-bromopicrotoxinic acids, but shown by crystallographic examination to be different from either. (We are indebted to Mr. J. J. Reed, Petrologist to the Geological Survey of the New Zealand Department of Scientific and Industrial Research, for this and other optical examinations.) We suggest tentatively that the new bromo-derivative and the corresponding acid are derived from an isomer of *neo*-picrotoxinin, formed by the alternative dehydration of picrotin to give the *iso*propenyl rather than the *iso*propylidene system :

$$\text{Picrotin, } C_{12}H_{11}O_6\cdot CMe_2\cdot OH \xrightarrow{-H_2O} \begin{cases} \rightarrow C_{12}H_{10}O_6{:}CMe_2, \textit{neo}\text{Picrotoxinin} \\ \rightarrow C_{12}H_{11}O_6\cdot CMe{:}CH_2\ ? \end{cases}$$

It appears, therefore, that picrotin is related to *neo*picrotoxinin rather than to picrotoxinin. This may be correlated with previous observations that picrotin and *neo*picrotoxinin can be acetylated, whereas picrotoxinin cannot (under comparable conditions), and that picrotin and *neo*picrotoxinin with hot dilute mineral acid yield picrotonol whereas picrotoxinin yields picrotoxic acid.

Sutter and Schlittler obtained succinic acid on permanganate oxidation of picrotin, picrotin acetate, or picrotoxinin; if this is formed directly, it indicates the existence of the fragment $\cdot CH_2\cdot CH_2\cdot$ in these substances. The oxidations, however, were carried out after the compounds had been heated at *ca.* 90° with sodium carbonate solution, which would presumably cause extensive degradation to (II), etc.; the oxidation products isolated might then be largely derived from these artefacts. Although (II), the common product isolated from all alkaline degradation mixtures so far investigated, does not admit of oxidation to succinic acid, its deoxy-derivative (IV) has been obtained in (relatively) high yield by degradation of picrotoxinin with hot barium hydroxide solution (Sutter and Schlittler, *loc. cit.*, 1949), and the succinic acid may have arisen from this source. No succinic acid was isolated on oxidation of dihydropicrotoxinin; in this experiment the preliminary heating with carbonate solution was to 20° only and the isolation of dihydropicrotoxic acid from the oxidation reaction shows that degradation had not proceeded so far as in the other cases.



(IV)

Experimental

Alkaline Degradation of Dihydropicrotoxinin.—Finely powdered dihydropicrotoxinin (5 g.) was dissolved in 10% sodium carbonate solution (730 ml.) at 80° while being agitated with a stream of nitrogen (30 minutes). The mixture, after continuous ether-extraction for 24 hours, was acidified with concentrated hydrochloric acid to pH *ca.* 6 and extracted with ether for 14 hours (extract A). Further 6-hour extractions were made until no more material was removed (extracts B). The aqueous layer was then acidified to Congo-red and extracted for 12 hours (extract C). Extract A was dried (Na_2SO_4), evaporated, and kept overnight in the refrigerator. A semicrystalline product was obtained from which resinous material was removed with a little ether, leaving white crystals (500 mg.) of δ-hydroxy-γ-keto-β-*iso*propylvaleric acid (I). A further quantity (200 mg.) was obtained by working up the ethereal mother-liquor. Recrystallised from ether–light petroleum (b. p. 40—60°), it had m. p. 110—110·5° (Found : C, 54·8; H, 7·9. Calc. for $C_8H_{14}O_4$: C, 55·2; H, 8·1%), $\lambda_{max.}$ 279 mμ (log ε = 1·56). Extracts B yielded oils which were not further investigated, although no doubt they contained

much of the lactone of the acid. Extract C, worked up in the same way as extract A, gave 5-hydroxy-3-keto-2-methyl*cyclo*pent-1-ene-1-carboxylic acid (210 mg.), crystallising from ether in bundles of small needles, m. p. 156° (Found: C, 53·5; H, 5·1. Calc. for $C_7H_8O_4$: C, 53·8; H, 5·2%), $\lambda_{max.}$ 234 (log ε = 4·02) and 332 mμ (log ε = 1·57).

Separation of Acids (I) *and* (II) *by Ion-exchange.*—A mixture of (I) (*ca.* 150 mg.) and (II) (*ca.* 140 mg.) in water (20 ml.) was passed down a column of "De-Acidite B" (20 × 1/2"), being followed by water. The first 20 ml. were returned to the top of the column and 90 ml. then collected and ether-extracted, to yield (I) (130 mg.). After the column had been washed with a further quantity of water (150 ml.), 10% sodium carbonate solution was passed through and 90 ml. were collected and worked up, to yield (II) (110 mg.).

Dihydroneopicrotoxinin.—Picrotoxin (6 g.) in alcohol was hydrogenated in the presence of palladium chloride (0·3 g.) dissolved in dilute hydrochloric acid (from 6 drops of concentrated acid) until no more hydrogen was absorbed (48 hours). Alcohol was removed and the product heated under reflux with 3% sulphuric acid (300 ml.) for 18 hours. Large plate-like crystals (0·57 g.) separated from the cold solution, and a further quantity (0·91 g.) was obtained by addition of an excess of sodium hydrogen carbonate and extraction with ethyl acetate. The product had m. p. 256° (from alcohol).

Alkaline Degradation of Dihydroneopicrotoxinin.—Finely ground dihydro*neo*picrotoxinin (1·5 g.) was heated with 10% sodium carbonate solution (80 ml.) at 90° with agitation by nitrogen. After 25 minutes the reaction mixture was cooled and ether-extracted for 24 hours (nothing was extracted). The reaction mixture was then made faintly acid to Congo-red; the yellow colour of the solution faded as did that of the solution obtained from dihydropicrotoxinin. The solution was again ether-extracted for 24 hours and on evaporation of the dried extract a light yellow oil remained. When seeded with the acid (I), this yielded crystalline (I) (21 mg.), m. p. 109°, mixed m. p. 110°. The aqueous solution was then acidified more strongly and again ether extracted for 24 hours, to yield finally a light yellow oil (105 mg.) which when seeded with the acid (II) deposited crystals, m. p. 152°, mixed m. p. with (II), 154°. The identities of the degradation products were confirmed by comparison of the absorption spectra.

Alkaline Degradation of Picrotin.—Finely powdered picrotin (5 g.) was heated with 10% sodium carbonate solution (730 ml.) at 70—75° with agitation by nitrogen. After 30 minutes the amber-coloured solution was steam-distilled (no acetone was detected), the colour changing to orange. The resulting solution was ether-extracted for 12 hours, to yield a little unchanged picrotin. Ether-extractions were then made at pH 6·5 (a little oil), pH 5 (a red oil, 1·89 g.) and pH 3 (some oil, finally yielding crystals of acid (II) (mixed m. p. and identical absorption spectra). The red oil was dissolved in ether and chromatographed on alumina. The ether was followed by ether containing 10% of methanol, ether containing 50% of methanol, and finally methanol. A little white solid material was isolated from the final methanolic elution, the other extracts yielding brown and (mainly) red oils. An attempt to crystallise the above-mentioned white solid from alcohol gave only a red oil, insoluble in ether. However, when dissolved in dilute sodium hydroxide solution and then acidified, it could again be extracted with ether. A crystalline specimen was not obtained, the acid forming the ether-insoluble lactone, even below 90°.

Dehydration of the Sodium Salt of the "True Picrotinic Acid."—Picrotin (2 g.), dissolved in 2% sodium hydroxide solution, was titrated with acid until only just alkaline and the water removed under reduced pressure from a water-bath at 30—40°. A white glassy solid remained, which could be ground to a powder but on exposure to the air rapidly became sticky. This was ground with fused oxalic acid (2 g.) and the mixture heated at 110° for 24 hours. The resulting brown cake was dissolved in boiling water and a brown supernatant oil removed (crude picrotin?). The solution was filtered and treated with bromine water. The white precipitate which formed coagulated into a few clots which were filtered off and crystallised from ethyl alcohol several times, to give needles, m. p. 264° alone or mixed with authentic bromo*neo*picrotoxinin of the same m. p. The yield was only 3—4 mg.

Dehydration of Picrotin.—On account of the small yield of bromo-derivative obtained in the dehydration experiments special care was taken to remove all traces of bromopicrotoxinin from the picrotin used. This is best done by dissolving the picrotin in cold dilute alkali and filtering off any insoluble material. Acidification regenerates very pure picrotin. Picrotin (1 g.) was added to oxalic acid (2 g.) melted in a test-tube. The resulting clear colourless solution was kept just at the f. p. for about 30 minutes, then cooled and dissolved in hot water. On addition of bromine water a white precipitate appeared which was filtered off, washed with dilute alkali to remove unchanged picrotin and oxalic acid, and then crystallised from ethyl

alcohol as small cubes, m. p. 264° (*ca.* 5 mg.). The alcoholic mother-liquors gave bromo*neo*-picrotoxinin.

The former substance (*ca.* 5 mg.) was suspended in hot water (0·5 ml.), and dilute aqueous sodium hydroxide added dropwise until dissolution was complete. The product was cooled and acidified carefully, yielding a white precipitate which, crystallised from water, had m. p. *ca.* 250°. This acid has α 1·532 ± 0·001, β = 1·558 ± 0·001, and γ = 1·562 ± 0·001, the measurements being made with sodium light. Petrographic examination of α-bromopicrotoxinic acid was hindered because of the fine needle-like crystals in which it separates, but the following measurements were made: α = 1·546 ± 0·002, γ = 1·549 ± 0·002. When samples of the new acid and α-bromopicrotoxinic acid were immersed in a liquid of n_D 1·5472, the lowest refractive index (α) of α-bromopicrotoxinic acid was considerably higher, whereas that of the unknown was less. The highest refractive index (γ) of β-bromopicrotoxinic acid exceeded 1·583.

Appendix

Oxidation of Picrotin with Potassium Permanganate in Cold Alkaline Solution.—Picrotin (10 g.) was dissolved by heating it for 15 minutes at 90° with 15% sodium carbonate solution (1 l.). The reddish-yellow solution was then cooled to 0° and potassium permanganate (19 g.) in ice-cold water (950 ml.) added slowly with stirring during 1 hour. The mixture was then set aside for 16 hours. The manganese dioxide was brought into solution with sulphur dioxide, and the whole concentrated to 200 ml. at 40°, acidified with concentrated sulphuric acid to Congo-red, and ether-extracted for 24 hours. The ethereal solution was evaporated and the residue dissolved in water (10 ml.) and made alkaline with ammonia. Addition of an ammoniacal solution of calcium chloride precipitated oxalic acid as the calcium salt, which was filtered off, and the filtrate was again acidified and ether-extracted for 30 hours. The ethereal extract was dried (Na_2SO_4), evaporated, and then sublimed, with the following results: bath 100°/12 mm., sublimation temp. 60—80°, α-hydroxy*iso*butyric acid, m. p. 78—80° (0·332 g.); bath 120—160°/12 mm., succinic acid, m. p. 178·5—180°.

It is known that *iso*butyric acid itself can be oxidised by potassium permanganate to the α-hydroxy-acid. Experiments showed that this is possible only when the oxidation is carried out with heating. At 0° α-hydroxy*iso*butyric acid was not isolated.

Oxidation of Picrotin Acetate with Potassium Permanganate in Cold Alkaline Solution.—Picrotin acetate (4 g.) and 15% sodium carbonate solution (400 ml.) were heated for 15 minutes on the water-bath. The resulting solution was then oxidised at 0° with potassium permanganate (12·5 g.) in water (500 ml.). When worked up as above, the product yielded succinic acid but no α-hydroxy*iso*butyric acid.

Oxidation of Picrotoxinin with Potassium Permanganate in Cold Alkaline Solution.—Picrotoxinin (5 g.) was dissolved in 1% sodium carbonate solution (500 ml.) at 90° (5 minutes) (no acetone formed) and then cooled to 0°. Potassium permanganate (16·25 g.) in ice-cold water (650 ml.) was added with stirring. The mixture was clarified with sulphur dioxide, made slightly alkaline, concentrated *in vacuo* to 100 ml., and continuously extracted with ether (no residue on evaporation). The aqueous solution was then acidified with sulphuric acid to Congo-red and ether-extracted for 48 hours. The ether on evaporation left a syrupy residue containing oxalic acid, which was removed as the calcium salt (cf. above). The resulting ammoniacal solution was acidified and ether-extracted and the syrup remaining after evaporation of the ether sublimed at 120—130° (bath)/0·1 mm. The solid sublimate was transformed into the calcium salt, and again traces of the sparingly soluble oxalate separated. The syrup, which was now free from oxalic acid, was treated with acetone, pure succinic acid being obtained (0·27 g.), with m. p. 181—182° (phenylhydrazine salt, m. p. 95—96°).

Oxidation of Dihydropicrotoxinin with Potassium Permanganate in Alkaline Solution at 50°.—Dihydropicrotoxinin (2 × 5 g.) was dissolved in 1% sodium carbonate solution (2 × 1 l.) at 20°. Powdered potassium permanganate (2 × 22 g.) was then added and the solutions were heated to 55° for 19 hours with stirring. The vessel was connected to a trap cooled to −80°. The mixture was clarified with sulphur dioxide and made strongly alkaline and distilled in steam for a short time. Acetaldehyde was isolated as the 2 : 4-dinitrophenylhydrazone, m. p. 156—158° (Found: C, 43·1; H, 3·5; N, 24·5. Calc. for $C_8H_8O_4N_4$: C, 42·9; H, 3·6; N, 25·0%) (yield, as aldehyde, 0·12 g.). The steam-distilled alkaline solution was concentrated *in vacuo* to 500 ml. and extracted with ether. This extract left no residue. The aqueous solution was acidified with sulphuric acid (cooling) to Congo-red and ether-extracted for 3 days. The oil remaining after evaporation of the ether yielded, as above, 3·39 g. of pure oxalic acid. The

ammoniacal solution was acidified with sulphuric acid to Congo-red and extracted with ether. The extract yielded dihydropicrotoxic acid (0·95 g.), m. p. 253—254° (Found: C, 57·8; H, 6·4. Calc. for $C_{15}H_{20}O_7$: C, 57·7; H, 6·4%), and a viscous reddish-yellow oil. This oil gave the following fractions at 12 mm.: b. p. 53—54°, converted into *p*-phenylphenacyl *iso*butyrate, m. p. 84·5—85·5° (yield, as acid, 65 mg.); b. p. 88—90° (no crystalline *p*-phenylphenacyl ester); b. p. 108—110° (*p*-phenylphenacyl ester, m. p. 108—140°); b. p. 128—129° (no crystalline *p*-phenylphenacyl ester); and b. p. from 130°, a red-brown glassy mass, which after esterification (diazomethane) gave on distillation at 12 mm. fractions of b. p. 78—80°, 90—104°, and 125—128° respectively.

Acetaldehyde and *iso*butyric acid were also isolated when dihydro-α-picrotoxinic acid was oxidised with potassium permanganate.

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