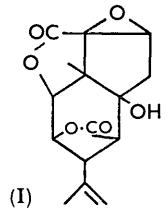
738. *Picrotoxin. Part V.**

By J. S. E. Holker, K. U. Holker, A. McGookin, Alexander Robertson, K. Sargeant, and (in part) D. E. Hathway.

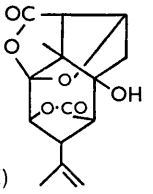
Reduction of picrotoxinin with lithium aluminium hydride gave two products which have been inter-related by oxidation with periodic acid. Similar transformations of α-dihydropicrotoxinin and picrotin have been carried out and direct structural relations established for the reduction products. The resulting evidence is discussed with particular reference to the structures (I), (II), and (III) which other workers [1,2] have put forward for picrotoxinin.

The reduction of picrotoxinin, β-bromopicrotoxinin, and β-bromopicrotoxinic acid and its ester with lithium aluminium hydride has recently been described by Johns, Slater, and Woods [1] who on the basis of their results have tentatively modified the structure (I) for picrotoxinin proposed by Conroy [3] to (II) or (III). In continuation of earlier work * an investigation on the reduction of picrotoxinin and picrotin with the same reagent, which was initiated in these laboratories in 1950, appears to be somewhat more extensive than that of Johns *et al.* and the interpretation of the results differs considerably from theirs.

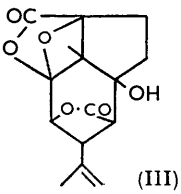
With this reagent we have found that picrotoxinin ($C_{15}H_{16}O_6$) gave a mixture of two water-soluble compounds $C_{15}H_{20}O_6$ and $C_{15}H_{22}O_6$ in 30% and 3% yield, designated



(I)



(II)



(III)

compound A and compound B respectively. The infrared absorption spectra of (A) and (B) measured in mineral oil mull were similar and both showed bands between 3550 and 3300 cm.$^{-1}$ (OH), a band at 1650 cm.$^{-1}$ (double bond of the *iso*propenyl system), and a total absence of absorption between 1850 and 1700 cm.$^{-1}$, indicating an absence of carbonyl functions.

Compound A showed some acidic properties and it could not be extracted with solvents from dilute aqueous sodium hydroxide. It formed a monomethyl ether ($C_{16}H_{22}O_6$) on treatment with dimethyl sulphate and sodium hydroxide, a diacetyl derivative ($C_{19}H_{24}O_8$), a 2 : 4-dinitrophenylhydrazone ($C_{21}H_{24}O_9N_4$), and a dihydro-derivative ($C_{15}H_{22}O_6$) on hydrogenation in the presence of Adams catalyst. Dihydro-compound A also showed some acidic properties and formed a monomethyl ether ($C_{16}H_{24}O_6$), a diacetyl derivative ($C_{19}H_{26}O_8$), and a 2 : 4-dinitrophenylhydrazone ($C_{21}H_{26}O_9N_4$). By the Zerewitinoff method compound A and its dihydro-derivative appear each to contain three active hydrogen atoms. The dihydro-derivative of compound A monomethyl ether was also formed by hydrogenation of compound A monomethyl ether with an Adams catalyst. The monomethyl ethers of compound A and its dihydro-derivative, which do not show acidic properties and cannot be acetylated even under drastic conditions, formed mono-2 : 4-dinitrophenylhydrazones identical with the respective hydrazones from compound A and its dihydro-derivative, reactions involving the demethylation of the ethers under the acidic conditions employed. This was confirmed by the hydrolysis of dihydro-compound A

* Part IV, *J.*, 1939, 1261.

[1] Johns, Slater, and Woods, *J.*, 1956, 4715.
[2] Conroy, *J. Amer. Chem. Soc.*, 1951, **73**, 1889.
[3] Bellamy, "The Infra-red Spectra of Complex Molecules," Methuen, London, 1954, p. 128.

monomethyl ether with acid to give dihydro-compound A in high yield. The reduction of α-dihydropicrotoxinin with lithium aluminium hydride gave dihydro-compound A, but a product corresponding to compound B was not isolated.

In view of the complete absence of carbonyl bands in the infrared absorption spectra of compound A, dihydro-compound A, and their respective methyl ethers, the formation of 2 : 4-dinitrophenylhydrazones can only be explained on the assumption that these compounds contain a system which generates an aldehydic or ketonic group under the acid conditions occurring in the preparation of the 2 : 4-dinitrophenylhydrazone, a conclusion in accordance with our inability to prepare semicarbazones of these compounds under the usual slightly alkaline conditions. These results, together with the ready formation and acid fission of the monomethyl ethers of compound A and its dihydro-derivative, are readily explained on the view that these products contain the hemiacetal system $\geq$C(OH)·O· and the monomethyl ethers the acetal system $\geq$C(OMe)·O·. The formation of the diacetate of compound A under conditions in which picrotoxinin is not acetylated implies that, in addition to that of the hemiacetal system, a second hydroxyl group has been generated in the reduction with lithium aluminium hydride.

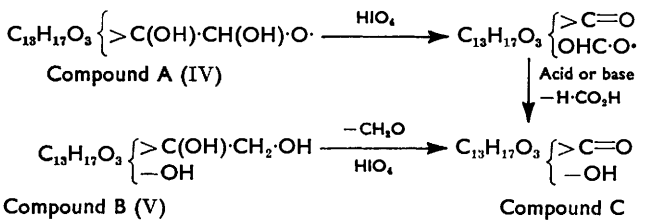
Compound B formed a diacetate ($C_{19}H_{26}O_8$) but did not show acidic properties; it did not form a methyl ether or a 2 : 4-dinitrophenylhydrazone. By the Zerewitinoff method, it appeared to contain four active hydrogen atoms.

Compound A and dihydro-compound A reacted with one molecular equivalent of periodic acid, giving products $C_{15}H_{18}O_6$ and $C_{15}H_{20}O_6$ respectively. Since these derivatives could not be acetylated it appears reasonably certain that the two hydroxyl groups of compound A and dihydro-compound A, which can be acetylated, have been destroyed in the oxidation and consequently are probably present in an α-glycol residue. On hydrogenation the oxidation product from compound A gave a dihydro-derivative identical with the oxidation product from dihydro-compound A. Further, each oxidation product gave positive hydroxamic acid tests for esters and on mild acid or alkaline hydrolysis generated formic acid together with the corresponding deformyl derivative, $C_{14}H_{18}O_5$ and $C_{14}H_{20}O_5$ respectively, which we have named compound C and dihydro-compound C. These products are readily reconverted into formates identical with the parent esters and similarly gave monoacetates ($C_{16}H_{20}O_6$) and ($C_{16}H_{22}O_6$) respectively; but, whereas dihydro-compound C, gave a crystalline 2 : 4-dinitrophenylhydrazone ($C_{20}H_{24}O_8N_4$), the corresponding derivative from compound C was amorphous. Compound C was also obtained along with formaldehyde by the oxidation of compound B with periodic acid; the monomethyl ether of compound A or its dihydro-derivative did not react with this reagent. It thus appears that in the oxidation products from compound A and its dihydro-derivative a ketonic carbonyl and an *O*-formyl group are generated. The infrared absorption spectra of both products $C_{15}H_{18}O_6$ and $C_{15}H_{20}O_6$ (in $CHCl_3$) show bands at 1757 and 1724 cm.$^{-1}$; in addition the product from compound A had a band at 1643 cm.$^{-1}$ (·CMe:CH_2). Compound C and dihydro-compound C (in $CHCl_3$) show a band at 1745 cm.$^{-1}$, and the former compound an additional band at 1643 cm.$^{-1}$ (·CMe:CH_2). The band at 1724 cm.$^{-1}$ exhibited by the oxidation products is due to the formate ester group, and that at 1757 cm.$^{-1}$ must be due to a ketonic group in a strained ring system.[3] The shift of this band to 1745 cm.$^{-1}$ in compound C and its dihydro-derivative is probably due to hydrogen bonding between the generated hydroxyl group and the keto-group.[4] These results are satisfactorily explained if compound A and its dihydro-derivative contain an α-hydroxy-hemiacetal system >C(OH)·CH(OH)·O·C$\leq$, and compound B the system (V). The reaction sequence may then be represented by the annexed partial formulæ.

The reduction of dihydro-compound C with lithium aluminium hydride or with hydrogen and an Adams catalyst gave a compound D, $C_{14}H_{22}O_5$, containing two additional hydrogen atoms and presumably arising by reduction of the keto- to a hydroxyl group. In agreement with this, compound D formed a diacetate ($C_{18}H_{26}O_7$) whereas dihydro-compound C

[4] Grove and Willis, *J.*, 1951, 877.

formed a monoacetate and, further, compound D did not show absorption in the carbonyl region of the infrared spectrum. With hydrogen and an Adams catalyst compound C absorbed two mols. of hydrogen, giving compound D, a reaction presumably involving saturation of the *iso*propenyl system in conjunction with the reduction of the keto-group.

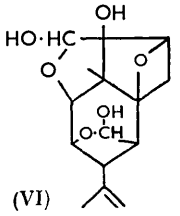


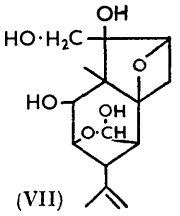
The reduction of compound C with lithium aluminium hydride furnished a product which is isomeric with dihydro-compound C, and apparently arising by reduction of the keto-group since on saturation of the *iso*propenyl group by catalytic hydrogenation this gave compound D.

The formation of an α-hydroxy-hemiacetal system (IV) in compound A and dihydro-compound A, and of the triol system (V) in compound B, seems to be best explained on the assumption that picrotoxinin and α-dihydropicrotoxinin contain an α-hydroxy-lactone or potential α-hydroxy-lactone system which undergoes reduction. Reduction of a lactone to a hemiacetal has been previously observed [5] in certain cases where a limited amount of lithium aluminium hydride has been employed. The isolation of an intermediate reduction product from picrotoxinin with an excess of lithium aluminium hydride is unusual but can be explained on the assumption that compound A is precipitated as an insoluble metallic complex and thus is preserved from further reaction. The reduction of the lactone system envisaged in the formation of compounds A and B only requires a hydrogen increment of two and four atoms respectively, whereas the compounds differ from the parent by four and six atoms respectively. Hence it appears that in the production of compound A and B a second function present in picrotoxinin must also have been reduced, involving the addition of two more hydrogen atoms. The nature of this second reducible function in picrotoxinin is less clear, but if the compound has a dilactone structure both lactone rings must be reduced in the formation of compound A and B, and, from the composition and nature of the products, reduction of the second lactone ring must be confined to the addition of two hydrogen atoms, *i.e.*, formation of a hemiacetal system would normally be expected. It has proved impossible to obtain direct evidence for the presence of this system in our transformation products. Thus by its method of preparation, dihydro-compound C, should still contain the second hemiacetal system together with a ketonic function and an alcoholic hydroxyl group. It forms only a monoacetate, showing infrared bands (in $CHCl_3$) at 1739 (*O*-acetate) and 1757 cm.$^{-1}$ (keto-group in a strained ring system) and a doublet at 3597 and 3425 cm.$^{-1}$, indicating that this compound still contains a hydroxyl group which may be present in a hemiacetal system. Many attempts were made to isolate an oxidation product from dihydro-compound C acetate containing a lactone ring regenerated by the oxidation of the hemiacetal system but, although the acetate readily consumed chromic oxide, the only homogeneous product isolated was unchanged starting material. Thus it appears that the oxidation products formed undergo further degradation more readily than the original compound. On the other hand, with chromic acid dihydro-compound C gave a product $C_{14}H_{18}O_5$ which apparently arises by oxidation of the secondary alcoholic hydroxyl group to a keto-group. In support of this conclusion the infrared spectrum of the oxidation product shows bands (in $CHCl_3$) at 1760 (keto-group in strained ring system) and 1718 cm.$^{-1}$ (attributed to the second keto-group formed in the oxidation and probably present in a

[5] Arth, *J. Amer. Chem. Soc.*, 1953, **75**, 2413; Hinder and Stoll, *Helv. Chim. Acta*, 1954, **37**, 1866.

six-membered ring) and a doublet at 3600 and 3450 cm.$^{-1}$ (hydroxyl). Therefore, the presence of a hemiacetal system in these compounds seems unlikely. Nevertheless, attempts were made to accommodate the foregoing reactions on the basis of the Conroy structure (I) for picrotoxinin on the assumption that in the formation of compound A the reduction of both lactone rings to hemiacetal systems is accompanied by a 1 : 2-epoxide–ether rearrangement. Compounds A and B would then have structures of the type (VI) and (VII) respectively.


(VI)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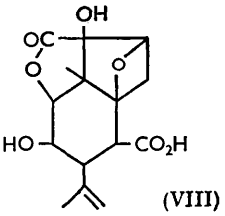
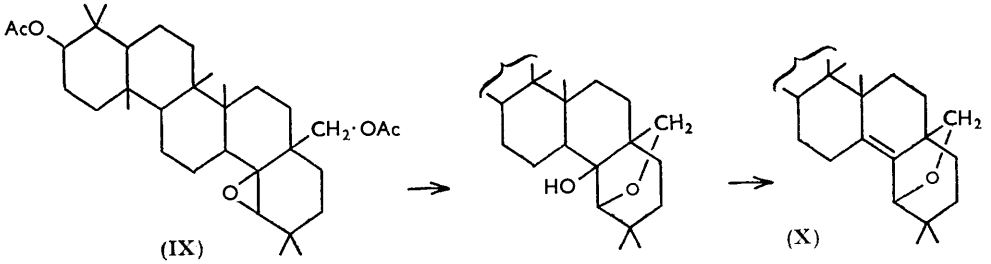
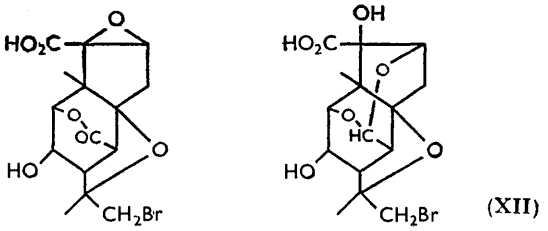

(VIII)

A similar rearrangement of a 1 : 2-epoxide to an ether in a lithium aluminium hydride reduction has been postulated [6] to explain the formation of compound (X) from moradiol diacetate oxide (IX); to account for the properties of picrotoxic acid Benstead *et al.*[7] have


(IX) (X)

suggested that it had structure (VIII) and that its formation from picrotoxinin involves epoxide–ether rearrangement.

After this communication * had been drafted and originally submitted for publication, a paper by Conroy [8] became available in which the reduction product of β-bromopicrotoxinic acid (XI) with sodium borohydride was shown to be an α-hydroxy-acid. On mechanistic grounds the structure (XII) was advanced for this product. This is attractive


(XI) (XII)

because it explains the reduction of the lactone ring of β-bromopicrotoxinic acid by the addition of two hydrogen atoms without the formation of a hemiacetal system. Similarly,

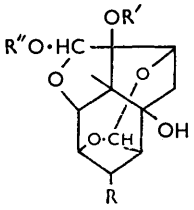
* In place of structure (VI), one of us (J. S. E. H.) originally considered that of three alternatives structure (XIII; R = ·CMe:CH_2, R′ = R″ = H) was possible for the reduction product. Following the paper by Dr. H. Conroy [8] this possibility, which was suggested independently by a referee, became more attractive.—A. R.

[6] Barton and Brooks, *J.*, 1951, 257.
[7] Benstead, Gee, Johns, Martin Smith, and Slater, *J.*, 1952, 2292.
[8] Conroy, *J. Amer. Chem. Soc.*, 1957, **79**, 1726.

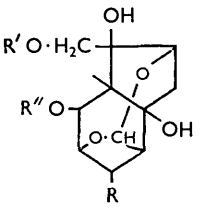
the foregoing reduction of picrotoxinin may be explained satisfactorily on the assumption that the formation of compounds A and B proceeds by a parallel route.

Thus it appears that (XIII; R = ·CMe:CH_2, R′ = R″ = H) and (XIV; R = ·CMe:CH_2, R′ = R″ = H) are more acceptable formulations of compound A and B respectively than formulæ (VI) and (VII).

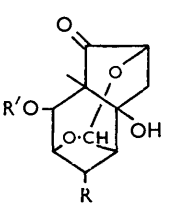
The acidic properties of compound A are considered to be due to the hemiacetal hydroxyl group of the α-hydroxy-hemiacetal system and accordingly the momomethyl ether is formulated as (XIII, R = ·CMe:CH_2, R′ = H, R″ = Me) and compound A diacetate as (XIII; R = ·CMe:CH_2, R′ = R″ = Ac). The reason for the failure of compound A monomethyl ether to undergo acetylation is obscure. The diacetyl derivative of compound B is represented by (XIV; R = ·CMe:CH_2, R′ = R″ = Ac), the periodic acid



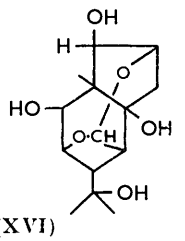
(XIII)



(XIV)



(XV)



(XVI)

product from compound A by (XV; R = ·CMe:CH_2, R′ = CHO), and compound C by (XV; R = ·CMe:CH_2, R′ = H).

By the reduction of β-bromopicrotoxinin ($C_{15}H_{15}O_6Br$) with lithium aluminium hydride Johns *et al.*[1] have obtained two products $C_{15}H_{23}O_6Br$ and $C_{15}H_{21}O_6Br$, of which compound $C_{15}H_{23}O_6Br$ reacts with one molecular equivalent of periodate in neutral or alkaline solution and two equivalents in acid solution and, in both acid and alkaline periodate oxidations, " one equivalent of back-titratable acidity " is produced. These authors attribute this acidity to the formation of a lactone ring in the oxidation product, but in view of the present results the acidity is in all probability due to a formate ester group and therefore structures (II) and (III) for picrotoxinin, which are based on the presence of a lactone ring in this periodate product, do not merit further consideration at this stage.

In an examination of the reduction of picrotin ($C_{15}H_{18}O_7$) with lithium aluminium hydride a water-soluble compound ($C_{15}H_{22}O_7$) was obtained which formed a 2 : 4-dinitrophenylhydrazone and a diacetate ($C_{19}H_{26}O_9$) under conditions in which picrotin is not acetylated. This reduction product corresponds with compound A from picrotoxinin and is formulated as (XIII; R = ·CMe_2·OH, R′ = R″ = H). Though the residues left after the isolation of (XIII; R = ·CMe_2·OH, R′ = R″ = H) gave some formaldehyde on oxidation with periodic acid and therefore probably contained small amounts of a substance corresponding to compound B, this product could not be isolated. Unlike compound A, compound (XIII; R = ·CMe_2·OH, R′ = R″ = H) did not show acidic properties and did not give a methyl ether. It reacted with an equimolecular proportion of periodic acid to give a mixture of the formate (XV; R = ·CMe_2·OH, R′ = CHO) and the deformyl compound (XV; R = ·CMe_2·OH, R′ = H). The formate, which could not be acetylated, was readily hydrolysed to the deformyl compound, which, like compound C, yielded a monoacetate (XV; R = ·CMe_2·OH, R′ = Ac) and a 2 : 4-dinitrophenylhydrazone.

A direct relationship has been established between compound C of the picrotoxinin series and compound (XV; R = ·CMe_2·OH, R′ = H) derived from picrotin, confirming the earlier evidence for the structural similarity of picrotoxinin and picrotin. Thus, treatment of compound C with perbenzoic acid gave an epoxide which was not isolated but subjected immediately to reduction with lithium aluminium hydride, giving a compound ($C_{14}H_{22}O_6$) formulated as (XVI). This product, which was formed from compound (XV;

R = ·CMe_2·OH, R′ = H) by reduction with lithium aluminium hydride or by hydrogenation in the presence of a Raney nickel catalyst, surprisingly formed a triacetate.

Although the work described in this paper can be rationally explained on the Conroy picrotoxinin formulation it does not necessarily exclude other possible structures and further work is in progress.

Experimental

Unless stated otherwise optical rotations were measured in EtOH at room temperature (18—22°) with a 1-dm. tube, ultraviolet absorption spectra in 95% EtOH with a Unicam spectrophotometer, and infrared spectra with a Perkin-Elmer model 21 double-beam spectrophotometer.

The light petroleum employed had b. p. 60—80°.

Reduction of Picrotoxinin.—A solution of lithium aluminium hydride (5 g.) in ether (500 ml.) was heated under reflux in the boiling flask of a Soxhlet apparatus, the thimble of which contained picrotoxinin (10 g.). 24 Hr. later the extraction was complete and, after the destruction of the excess of lithium aluminium hydride with methanol (20 ml.) in ether (150 ml.), ice (100 g.) and 2N-sulphuric acid (300 ml.) were added to the stirred mixture. The ether layer was separated, the aqueous phase was continuously extracted with ether for 20 hr., and the combined ether extracts were concentrated (50 ml.), diluted with ethyl acetate (600 ml.), dried ($MgSO_4$), and again concentrated (20 ml.). On being kept for several weeks the solution deposited colourless crystals (4·6 g.), m. p. 185—195°. The mother-liquors contained only intractable gum (3·7 g.). Repeated recrystallisation of the crystalline product from ethyl acetate gave compound A (XIII; R = ·CMe:CH_2, R′ = R″ = H) in hard rhombs (3·3 g.), m. p. 209—211°, $[\alpha]_D$ −29° (*c* 1·60) (Found: C, 60·7; H, 7·0; active H, 1·06. Calc. for $C_{15}H_{20}O_6$: C, 60·8; H, 6·8; 3 active H, 1·02%), which appears to be identical with the compound $C_{15}H_{20}O_6$, m. p. 212°, isolated by Johns *et al.*[1] Prepared by acetic anhydride–pyridine, the *diacetate* (XIII; R = ·CMe:CH_2, R′ = R″ = Ac) separated from benzene in thin plates, m. p. 213—214° (Found: C, 60·1; H, 6·4; Ac, 19·1. $C_{15}H_{18}O_6Ac_2$ requires C, 60·0; H, 6·4; Ac, 22·6%). The mother-liquors from A were evaporated and, after being extracted with boiling chloroform (5 × 25 ml.), the residue was crystallised from the same solvent, giving *compound* B (XIV; R = ·CMe:CH_2, R′ = R″ = H) in needles, m. p. 214—216°, which then formed hard rods (0·3 g.), m. p. 212—214° (from ethyl acetate–benzene), $[\alpha]_D$ −53° (*c* 1·11) (Found: C, 60·8; H, 7·2; active H, 1·3. $C_{15}H_{22}O_6$ requires C, 60·4; H, 7·4; 4 active H, 1·3%), mixed m. p. with compound A, 185—195°, The *diacetate* (XIV; R = ·CMe:CH_2, R′ = R″ = Ac) separated from benzene–light petroleum in needles, m. p. 160—162° (Found: C, 59·6; H, 6·7; Ac, 23·9. $C_{15}H_{20}O_6Ac_2$ requires C, 59·7; H, 6·8; Ac, 22·5%). Prepared with a solution of 2 : 4-dinitrophenylhydrazine sulphate in 6N-sulphuric acid, the *dinitrophenylhydrazone* of compound A separated from ethanol in yellow needles, m. p. 298—300° (decomp.) (Found: C, 52·9; H, 5·3; N, 11·7. $C_{21}H_{24}O_9N_4$ requires C, 52·9; H, 5·1; N, 11·8%).

Prepared by methyl sulphate–aqueous sodium hydroxide, the monomethyl ether (XIII; R = ·CMe:CH_2, R′ = H, R″ = Me) of compound A separated from benzene–light petroleum in needles, m. p. 170—171°, $[\alpha]_D$ +12° (*c* 2·38) (Found: C, 62·0; H, 7·2; OMe, 10·2. Calc. for $C_{16}H_{22}O_6$: C, 61·9; H, 7·2; OMe, 10·0%) (cf. Johns *et al.*[1] who give m. p. 170° for the ether prepared by this method and m. p. 176° for derivative prepared with diazomethane). With 2 : 4-dinitrophenylhydrazine sulphate in 8N-sulphuric acid the ether gave the 2 : 4-dinitrophenylhydrazone of compound A, separating from alcohol in yellow needles, m. p. and mixed m. p. 298—300° (decomp.) (Found: C, 52·8; H, 5·1; N, 11·7%; OMe, 0). This ether was recovered unchanged after treatment with (*a*) acetic anhydride and fused sodium acetate at the reflux temperature for 3 hr., (*b*) lithium aluminium hydride, (*c*) periodic acid, or (*d*) 10% aqueous potassium hydroxide at 100° for 3 hr.

Dihydro-compound A (XIII; R = ·$CHMe_2$, R′ = R″ = H).—α-Dihydropictotoxinin (10 g.) was reduced with lithium aluminium hydride (5 g.) by the procedure employed for picrotoxinin, and the gummy product (8·5 g.) was repeatedly recrystallised from benzene–ethyl acetate, giving dihydro-compound A in felted needles (3·2 g.), m. p. and mixed m. p. 209—211°, $[\alpha]_D$ −9·0° (*c* 1·43), identical with a specimen prepared by the reduction of compound A with hydrogen and a platinum catalyst (cf. Johns *et al.*[1] who do not give a m. p.) (Found: C, 60·4; H, 7·4; active H, 0·98. Calc. for $C_{15}H_{22}O_6$: C, 60·4; H, 7·4; 3 active H, 1·01%). The

mother-liquors contained only intractable gum. The *diacetate* (XIII; R = ·$CHMe_2$, R′ = R″ = Ac) separated from benzene–carbon tetrachloride in plates, m. p. 216—218° (Found: C, 59·6; H, 6·7; Ac, 19·1. $C_{19}H_{26}O_8$ requires C, 59·7; H, 6·8; Ac, 22·5%). The 2 : 4-dinitrophenylhydrazone was obtained as an amorphous yellow solid.

Methyl Ether of Dihydro-compound A (XIII; R = $CHMe_2$, R′ = H, R″ = Me).—Dihydro-compound A was methylated with dimethyl sulphate and sodium hydroxide, to give the *methyl ether* which separated from benzene–light petroleum in needles, m. p. 179—180°, $[\alpha]_D$ + 28° (*c* 1·08) (Found: C, 61·6; H, 7·4; OMe, 10·2. $C_{16}H_{24}O_6$ requires C, 61·5; H, 7·7; OMe, 10·0%).

On reduction in alcohol with hydrogen (approx. 1 mol. absorbed) and a platinum catalyst the methyl ether of compound A gave a theoretical yield of the dihydro-derivative which formed needles, m. p. and mixed m. p. 179—180°, $[\alpha]_D$ +28° (*c* 3·77), from benzene–light petroleum (Found; OMe, 9·9%). Treatment of this ether with 2 : 4-dinitrophenylhydrazine sulphate in 8N-sulphuric acid gave an amorphous, yellow 2 : 4-dinitrophenylhydrazone. The ether was recovered unchanged after being heated with boiling acetic anhydride and fused sodium acetate for 3 hr. or methyl iodide and silver oxide for 48 hr.

The ether (0·5 g.) was shaken with 2N-hydrochloric acid (50 ml.) at room temperature until dissolution was complete (24 hr.) and the resulting dihydro-compound A isolated with ether and crystallised from chloroform, forming felted needles (0·3 g.), m. p. 203—206°; admixed with an authentic specimen it had m. p. 204—208°.

Oxidation of Compound A with Periodic Acid.—Sodium periodate (4·4 g.) in water (20 ml.) and concentrated sulphuric acid (3·5 ml.) was added at 0° to a solution of compound A (4 g.) in water (60 ml.). The precipitate (3·13 g.), m. p. 162—165°, which began to separate after 30 sec. was collected 10 min. later and crystallised from benzene, giving the *product* (XV; R = ·CMe:CH_2, R′ = CHO) in plates or rhombs, m. p. 171—172°, $[\alpha]_D$ −121° (*c* 2·51), $\lambda_{max.}$ 309 mμ (ε 27) (Found: C, 61·4; H, 6·2; active H, 0·39. $C_{15}H_{18}O_6$ requires C, 61·2; H, 6·2; 1 active H, 0·34%). This compound gave a positive hydroxamic acid test for an ester and it was recovered unchanged after treatment with semicarbazide hydrochloride and sodium acetate in aqueous solution or with acetic anhydride and pyridine.

A solution of this oxidation product (2 g.) in 1% aqueous sodium hydroxide (35 ml.) was kept at room temperature for 2 days and then repeatedly extracted with ether. The dried ethereal solution was evaporated, leaving a colourless residue (1·71 g.), m. p. 170°, which on crystallisation from benzene gave *compound* C (XV; R = ·CMe:CH_2, R′ = H) in plates, m. p. 171—172°, $[\alpha]_D$ −178° (*c* 4·03), $\lambda_{max.}$ 312 mμ (ε 37); admixed with the parent compound it had m. p. 145—150° (Found: C, 63·1; H, 6·8; active H, 0·81. $C_{14}H_{18}O_5$ requires C, 63·1; H, 6·8; 2 active H, 0·75%). The concentrated residual alkaline liquors (10 ml.) were acidified with hydrochloric acid (5 ml.), and the formic acid present was characterised by conversion into benziminazole (0·49 g., 60%), m. p. and mixed m. p. 169—170°, giving the picrate, m. p. and mixed m. p. 225—226°, according to the method of Brown and Campbell.[9] Compound C gave a *monoacetate* (XV; R = ·CMe:CH_2, R′ = Ac) which separated from benzene in needles, m. p. 205—206° (Found: C, 62·3; H, 6·5; Ac, 13·9. $C_{16}H_{20}O_6$ requires C, 62·4; H, 6·6; Ac, 14·0%), and an amorphous yellow 2 : 4-dinitrophenylhydrazone.

Oxidation of Dihydro-compound A with Periodic Acid.—Dihydro-compound A (4 g.) was oxidised with periodic acid under the conditions used for the oxidation of compound A and the *product* (XV; R = ·$CHMe_2$, R′ = CHO) (3·2 g.), m. p. 180—186°, isolated from the reaction mixture and crystallised from benzene, forming plates or rhombs (2·7 g.), m. p. 187—188°, $[\alpha]_D$ −116° (*c* 3·09), $\lambda_{max.}$ 310·5 mμ (ε, 29) (Found: C, 60·8; H, 6·8; active H, 0·41. $C_{15}H_{20}O_6$ requires C, 60·8; H, 6·8; 1 active H, 0·34%). The same compound was obtained by reduction of the periodic acid oxidation product of compound A (2 g.) in alcohol with hydrogen (approx. 1 mol. absorbed) and a platinum catalyst and formed plates (1·05 g.), m. p. and mixed m. p. 187—188°, from benzene (Found: C, 61·1; H, 6·9%). The residues from the purification of this consisted of an inseparable mixture. In some (apparently identical) reduction experiments the only product isolated had m. p. 215—216·5° after purification from benzene and was later shown to be identical with compound D (see below.).

The periodic acid product from dihydro-compound A, like the corresponding product from compound A, gave a positive hydroxamic acid test for esters, and did not form an acetate or a semicarbazone.

Dihydro-compound C (XV; R = $CHMe_2$, R′ = H) (With J. H. Taylor).—The periodic acid

[9] Brown and Campbell, *J.*, 1937, 1699.

product (2·5 g.) from compound A was hydrolysed with 1% aqueous sodium hydroxide (40 ml.) at room temperature for 2 days and the resulting *dihydro-compound C* was isolated with ether and crystallised from benzene, forming plates, m. p. 187—188°, $[\alpha]_D$ −156° (*c* 3·00), $\lambda_{max.}$ 313 mμ (ε 24) (Found: C, 62·6; H, 7·5; active H, 0·89. $C_{14}H_{20}O_5$ requires C, 62·7; H, 7·5; 2 active H, 0·74%). Admixed with the oxidation product of dihydro-compound A, it had m. p. 145—150°. The formic acid produced in the hydrolysis was characterised by conversion into benziminazole, m. p. and mixed m. p. 169—170°.

On treatment with acetic anhydride, 95% formic acid, and pyridine dihydro-compound C regenerated the formate, m. p. 186—188°, identical with the periodic acid product from dihydro-compound A. It also gave a *monoacetate* (XV; R = $CHMe_2$, R′ = Ac), forming needles, m. p. 200°, from benzene (Found: C, 61·7; H, 7·2; Ac, 13·6. $C_{16}H_{22}O_6$ requires C, 61·9; H, 7·1; Ac, 13·8%), and a 2 : 4-*dinitrophenylhydrazone*, orange-yellow needles from alcohol, m. p. 246—250° (decomp.) (Found: C, 53·4; H, 5·3; N, 12·3. $C_{20}H_{24}O_8N_4$ requires C, 53·6; H, 5·4; N, 12·5%). The periodic acid oxidation product from dihydro-compound A gave the same 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 246—250°, when treated with a boiling solution of 2 : 4-dinitrophenylhydrazine (0·3 g.) and hydrochloric acid (0·5 ml.) in alcohol (25 ml.).

Dihydro-compound C (5 g.), in water (125 ml.), was treated at 0° with a solution of potassium dichromate (10 g.) in 8N-sulphuric acid (65 ml.) and 2 hr. later the crystalline precipitate was collected, washed, dried, and purified from benzene–ethyl acetate, giving the oxidation *product* in large rhombs (3·7 g.), m. p. 224—228° (decomp.), $[\alpha]_D$ −81° (*c* 2·79), $\lambda_{max.}$ 311 mμ (ε 128) (Found: C, 63·4; H, 6·8. $C_{14}H_{18}O_5$ requires C, 63·1; H, 6·8%).

The acetate of dihydro-compound C was treated with chromic oxide in acetic acid in many experiments performed under different conditions. Although reduction of the chromic oxide occurred readily at room temperature a crystalline oxidation product was not isolated.

iso*Dihydro-compound C*.—Compound C (4 g.) was reduced with lithium aluminium hydride (3 g.) in ether (150 ml.) by the method employed for picrotoxinin, and the product (3·92 g.), m. p. 178—180°, crystallised from benzene, giving iso*dihydro-compound C* in efflorescent needles, m. p. 179—180°, $[\alpha]_D$ −49° (*c* 3·47) (Found: C, 62·7; H, 7·4; active H, 1·16. $C_{14}H_{20}O_5$ requires C, 62·7; H, 7·5; 3 active H, 1·12%). The *diacetate* formed needles, m. p. 207—209°, from benzene–light petroleum (Found: C, 61·1; H, 6·7; Ac, 25·1. $C_{18}H_{24}O_7$ requires C, 61·3; H, 6·9; Ac, 24·5%).

Oxidation of Compound B with Periodic Acid.—(*a*) Sodium periodate (0·11 g.), in water (1 ml.) and sulphuric acid (2 drops), was mixed with a solution of compound B (0·1 g.) in water (2·5 ml.) at room temperature and 15 min. later the excess of periodic and iodic acid was removed by titration of the acidified solution with 0·4% sodium sulphite solution and a starch indicator. The mixture was diluted with water (60 ml.) and distilled. The formaldehyde in the distillate was characterised as the dimedone derivative (80 mg.), m. p. and mixed m. p. 188—189°. In another experiment the formaldehyde was isolated as the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 164° (decomp.).

(*b*) Compound B (0·5 g.), in water (20 ml.), was treated with a solution of sodium periodate (0·5 g.) in 4N-sulphuric acid (6 ml.) at room temperature and the product isolated with ether and crystallised from benzene, giving compound C in plates (0·26 g.), m. p. and mixed m. p. 171—172° (Found: C, 63·0; H, 6·9%).

Compound D.—(*a*) Dihydro-compound C (0·5 g.) was reduced with lithium aluminium hydride (1 g.) in ether, and the product (0·44 g.), m. p. 211—212°, isolated with ether and crystallised from benzene, giving *compound D* in plates, m. p. 215—216·5°, $[\alpha]_D$ −17° (*c* 3·85) (Found: C, 62·1; H, 8·4; active H, 1·02. $C_{14}H_{22}O_5$ requires C, 62·2; H, 8·2; 3 active H, 1·11%).

(*b*) Hydrogenation of compound C, dihydro-compound C, or *iso*dihydro-compound C (2 mol., 1 mol., and 1 mol. of hydrogen absorbed respectively) in alcohol with platinum catalyst gave a theoretical yield of compound D, m. p. and mixed m. p. 215—216·5°. The *diacetate* formed needles (from benzene–light petroleum), m. p. 201—202° (Found: C, 60·7; H, 7·1; Ac, 24·0. $C_{18}H_{26}O_7$ requires C, 61·0; H, 7·4; Ac, 24·3%).

Reduction of Picrotin.—This compound (8 g.) was reduced with lithium aluminium hydride (5 g.) in ether (500 ml.), and the resulting sticky resin triturated with cold ethyl acetate (30 ml.) to give an insoluble white powder (3 g.). Crystallised from ethyl acetate, this gave the reduction *product* (XIII; R = ·CMe_2·OH, R′ = R″ = H) in needles (2·5 g.), m. p. 222—224°, $[\alpha]_D$ −45°

(*c* 2·5) (Found: C, 57·5; H, 7·1. $C_{15}H_{22}O_7$ requires C, 57·3; H, 7·1%). This compound gave a *diacetate* which separated from ethyl acetate in rhombs, m. p. 242—244° (decomp.) (Found: C, 57·5; H, 6·4; Ac, 22·0. $C_{19}H_{26}O_9$ requires C, 57·3; H, 6·6; Ac, 21·6%), and a 2 : 4-*dinitrophenylhydrazone* from alcohol in yellow plates, m. p. 297—299° (decomp.) (Found: N, 11·6. $C_{21}H_{26}O_{10}N_4$ requires N, 11·3%).

Oxidation of the Foregoing Reduction Product.—(a) *With lead tetra-acetate.* A solution of the reduction product (1 g.) in acetic acid (5 ml.) was added to lead tetra-acetate (1·3 g.) in acetic acid (25 ml.), kept at room temperature for 24 hr., diluted with water (100 ml.), and neutralised with sodium hydrogen carbonate. Isolated with ether, the resulting formate (XV; R = CMe_2·OH, R′ = CHO) crystallised from acetone–light petroleum in long plates (0·6 g.), m. p. 187—189°, $[\alpha]_D$ −130° (*c* 4·7) (Found: C, 57·6; H, 6·9. $C_{15}H_{20}O_7$ requires C, 57·7; H, 6·5%).

(b) *With periodic acid.* A solution of the reduction product (4 g.) in water (15 ml.) was added to sodium periodate (5 g.) dissolved in water (10 ml.) containing sulphuric acid (60 drops) at 0° and the mixture kept at this temperature for 1 hr. The crystalline precipitate (2·5 g.), which on being heated melted at 80°, solidified, and then melted at 175—180°, was dehydrated at 100° and then crystallised from acetone–light petroleum, to give the formate in long plates (2 g.), m. p. and mixed m. p. 187—189° (Found: C, 57·5; H, 6·4%).

The residual aqueous solution left after the isolation of the formate was extracted into ether (5 × 50 ml.), and the extract washed with aqueous sodium hydrogen carbonate and dried (Na_2SO_4). Evaporation of the solvent left a white residue (1·5 g.) of the *deformyl compound* (XV; R = CMe_2·OH, R′ = H) which separated from acetone–light petroleum in cubes or from benzene–ethyl acetate in plates, m. p. 224—226°, $[\alpha]_D$ −175° (*c* 4·0) (Found: C, 59·2; H, 7·2. $C_{14}H_{20}O_6$ requires C, 59·1; H, 7·1%). The same compound was obtained by hydrolysis of the formate (0·2 g.) with sulphuric acid (15 drops) in water (20 ml.) at 0° for 24 hr. or with potassium hydroxide (0·2 g.) in water (7 ml.) at 0° for 1 hr. The production of formic acid in the hydrolysis of the oxidation product, m. p. 187—189° with 2N-sulphuric acid was established by isolation and conversion of the formic acid into benziminazole (yield, 47% of the theoretical for one formate residue). The deformyl compound, m. p. 224—226°, gave a *monoacetate* (XV; R = CMe_2·OH, R′ = Ac), forming needles, m. p. 222—223°, from acetone–light petroleum (Found: C, 59·2; H, 7·0; Ac, 15·8. $C_{16}H_{22}O_7$ requires C, 58·9; H, 6·8; Ac, 13·1%), and a 2 : 4-*dinitrophenylhydrazone*, yellow flakes, m. p. 278—280° (decomp.), from benzene–ethyl acetate (Found: C, 52·1; H, 5·1; N, 12·3. $C_{20}H_{24}O_9N_4$ requires C, 51·7; H, 5·1; N, 12·1%). The formate (XV; R = CMe_2·OH, R′ = CHO) gave the same 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 278—280° (decomp.) (Found: N, 12·1%), presumably by initial hydrolysis of the formate group by the acidic reagent.

Relationship between the Picrotoxinin and Picrotin Transformation Products.—(*a*) The deformyl compound (0·5 g.) derived from picrotin was reduced with lithium aluminium hydride (0·5 g.) in ether (100 ml.), and the product isolated by continuous extraction with ether and crystallised from benzene, giving the reduction *product* (XVI) in needles (0·3 g.), m. p. 80° (decomp.), resolidifying, and finally melting at 188—190°. Dried in a vacuum at 100° the compound had m. p. 188—190°, $[\alpha]_D$ −46° (*c* 2·6) (Found: C, 58·6; H, 7·9. $C_{14}H_{22}O_6$ requires C, 58·7; H, 7·8%). The same reduction product was formed by the hydrogenation of the deformyl compound (0·5 g.) in the presence of a Raney nickel (W. 7) catalyst, with an identical infrared absorption spectrum.

(*b*) Compound C (3·1 g.) from picrotoxinin was added to a solution of perbenzoic acid (2 mol. equivs.) in chloroform (100 ml.), and the mixture was kept in the dark at 0° for 48 hr., and filtered through a column of aluminium oxide (1 × 18 cm.) which was then washed with chloroform (100 ml.). Evaporation of the combined filtrate and washings left a gum which was reduced with lithium aluminium hydride (3 g.) in ether (100 ml.) by the continuous-extraction procedure. The product (1·8 g.) was isolated in ether and repeatedly crystallised from benzene, giving the compound, m. p. 188—190°, $[\alpha]_D$ −47° (*c* 2·82) (Found: C, 58·8; H, 7·6%), shown to be identical with the reduction compound (XVI) by mixed m. p. and by comparison of the infrared absorption spectra. The compound gave a *triacetate*, which separated from benzene–light petroleum in rhombs, m. p. 197—198° (Found: C, 58·2; H, 6·7; Ac, 31·4. $C_{20}H_{28}O_9$ requires C, 58·2; H, 6·9; Ac, 31·4%).

UNIVERSITY OF LIVERPOOL.

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