791. *Picrotoxin. Part VIII.*[1] *The Structures of α- and β-Picrotoxinones.*

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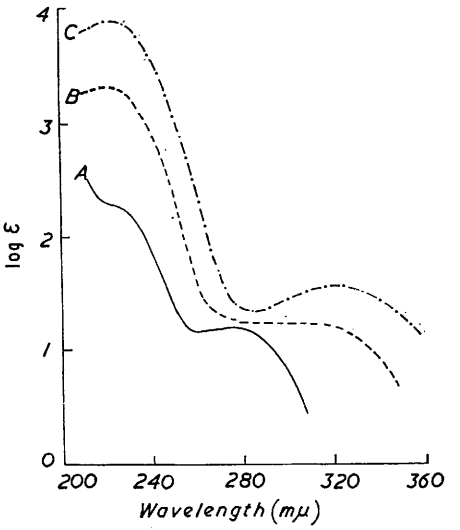
An investigation into the chemistry of α- and β-picrotoxinones has led us to propose structures (II) and (V) respectively for these compounds. A relation has been established between β-picrotoxinone and apopicrotoxinic dilactone (X).

It has been shown previously that ozonolysis of picrotoxinin, $C_{15}H_{16}O_6$, gives formaldehyde and a ketone, α-picrotoxinone, $C_{14}H_{14}O_7$,[2,3,4] which is isomerised to β-picrotoxinone when heated to 195° under reduced pressure or on prolonged treatment with boiling water.[2]

Despite the publication of much experimental work on α- and β-picrotoxinones and their derivatives,[2-8] the structures have never been defined. Since much of the published work is conflicting the problem has been re-investigated.



Absorption spectra of (A) α-picrotoxinone crystallised from warm methyl acetate and light petroleum (b. p. 40—60°), (B) α-picrotoxinone crystallised twice from ethyl acetate and light petroleum (b. p. 60—80°), and (C) β-picrotoxinone.

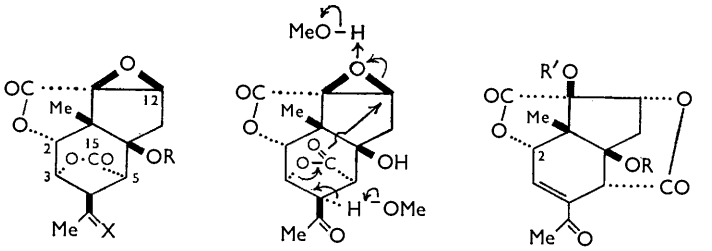
On the basis of the established structure[9,10] (I) for picrotoxinin, α-picrotoxinone should have structure (II). However, criticism of this type of structure has been made on the grounds that the ultraviolet absorption spectrum of α-picrotoxinone is not compatible with that of a compound containing an isolated ketonic carbonyl group.[6] We find that, when carefully purified from warm *methyl* acetate and light petroleum (b. p. 40—60°), α-picrotoxinone has $\lambda_{max.}$ 275 mμ (log ε 1·21) and gives a yellow 2,4-dinitrophenylhydrazone having $\lambda_{max.}$ 357 mμ (log ε 4·33). Although these facts are entirely compatible with a structure containing an isolated ketonic carbonyl group, it should be noted that when α-picrotoxinone is crystallised from boiling *ethyl* acetate and light petroleum (b. p. 60—80°) the ultraviolet absorption spectrum changes as shown in the Figure. This is

[1] Part VII, Holker, Robertson, and Taylor, *J.*, 1958, 2987.
[2] Horrmann, *Ber.*, 1916, **49**, 1554.
[3] Horrmann and Prillwitz, *Arch. Pharm.*, 1920, **258**, 200.
[4] Mercer and Robertson, *J.*, 1936, 288.
[5] Harland and Robertson, *J.*, 1939, 937.
[6] Slater, *J.*, 1949, 806.
[7] Johns, Slater, Woods, Brasch, and Gee, *J.*, 1956, 4715.
[8] Hathway, *J.*, 1957, 4953.
[9] Conroy, *J. Amer. Chem. Soc.*, 1951, **73**, 1889.
[10] Conroy, *J. Amer. Chem. Soc.*, 1957, **79**, 1726.

undoubtedly due to isomerisation of α- to β-picrotoxinone under the thermal conditions of the recrystallisation. Indeed, even our carefully purified samples of the α-isomer always show a shoulder at 222 mμ due to traces of the β-isomer. It seems reasonably certain that previous workers have measured the ultraviolet spectra of mixtures of the two isomers and therefore that their objections to structure (II) for α-picrotoxinone are invalid. This structure is supported by the infrared spectrum of α-picrotoxinone which shows bands at 3448 (tertiary OH), 1779 (γ-lactone), and 1709 cm.$^{-1}$ (ketonic C=O), and by the fact that the compound gave a positive iodoform reaction.[7]

In our experience the published methods [2] for the isomerisation of α- to β-picrotoxinone were inconvenient and gave rather low yields. We found that the β-isomer could be prepared in high yield by treating the α-isomer in methanol with a catalytic amount of sodium methoxide.

The structure of β-picrotoxinone followed from the properties of the compound and its reactions. Thus, the compound had $\lambda_{max.}$ 222 and 322 mμ (log ε 3·90 and 1·56) and gave a semicarbazone [3] having $\lambda_{max.}$ 274 mμ (log ε 4·36). These spectra are typical of αβ-unsaturated ketones and their semicarbazones respectively.[11] α-Picrotoxinone gave the same semicarbazone, presumably owing to isomerisation occurring under the alkaline conditions of the preparation. The generation of an αβ-unsaturated ketone system in the formation of β- from α-picrotoxinone (II) would be explicable in terms of dehydration of a β-hydroxy-ketone derived by opening the γ-lactone ring bridging $C_{(3)}$ and $C_{(5)}$ in α-picrotoxinone. Further, since it was known from previous results [10,12] that the lactonic carbonyl-oxygen atom at position 15 in picrotoxinin derivatives is suitably placed for displacement of the 12-epoxide grouping, it seemed likely that β-picrotoxinone had structure (V), formed from α-picrotoxinone in the methoxide-catalysed isomerisation by the mechanism shown in (IIa). The infrared spectrum of β-picrotoxinone had bands at 3528, 3460 (OH), 1774 (γ-lactone), 1743 (δ-lactone), and 1667 cm.$^{-1}$ (αβ-unsaturated ketone) in agreement with structure (V). Further, the presence of at least two hydroxyl groups in β-picrotoxinone was indicated since the compound formed a monoacetate which had



(I) R = H, X = CH_2
(II) R = H, X = :O
(III) R = Ac, X = :CH_2
(IV) R = Ac, X = :O

(IIa)

(V) R = R′ = H
(VI) R = H, R′ = Ac
(VII) R = Ac, R′ = H

hydroxyl absorption at 3510 cm.$^{-1}$. Ozonolysis of picrotoxinin acetate (III) gave α-picrotoxinone acetate (IV) which was readily isomerised by heat or by a trace of sodium methoxide to β-picrotoxinone acetate,* isomeric with the acetylation product from β-picrotoxinone. Since α-picrotoxinone acetate, unlike the two isomeric β-picrotoxinone acetates, did not show hydroxyl absorption in its infrared spectrum it is apparent that a

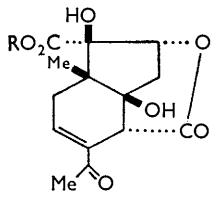
* In previous work on the ozonolysis of picrotoxinin acetate both α- and β-picrotoxinone acetate were isolated directly from the reaction.[8] This was probably due to hitherto unrecognised partial isomerisation during the isolation procedure.

[11] Gillam and Stern, "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," Arnold Ltd., London, 1954, pp. 91, 100.
[12] Holker, Holker, McGookin, Robertson, and Sargeant, *J.*, 1957, **3746**.

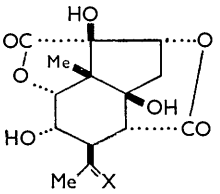
hydroxyl group is generated during the isomerisation reactions in agreement with the proposed mechanism. Thus, on the basis of structure (V) for β-picrotoxinone, the acetylation product is formulated as (VI), and the isomeric acetate from α-picrotoxinone acetate (IV) as (VII).

The observed *K*-band at 222 mμ in the ultraviolet absorption spectrum of β-picrotoxinone shows a considerable divergence from the usual figure of *ca.* 237 mμ for an αβ-disubstituted αβ-unsaturated ketone.[13] It is known, however, that an ester substituent allylic to the double bond of an αβ-unsaturated ketone causes a hypsochromic shift of the *K*-band, but the effect is not as great as that encountered in this case. Thus a 6α- or 6β-acetoxyl substituent in a 3-oxo-Δ^4-steroid lowers the *K*-band by up to 6 mμ.[14] In the case of β-picrotoxinone it seems likely that the allylic lactone bridge at $C_{(2)}$ would produce a similar shift and this, together with a further shift produced by the residual strain associated with the γ-lactone ring, might account for the position of the *K*-band.

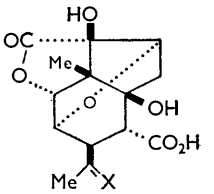
The allylic nature of the γ-lactone ring in β-picrotoxinone (V) has been demonstrated by hydrogenolysis of the compound to the monocarboxylic acid (VIII), characterised as its methyl ester (IX) and as the semicarbazone of the ester. The infrared spectra of the acid and its ester are in agreement with the proposed structures (see p. 4014). Moreover, the *K*-band at 233 mμ in the ultraviolet spectra of compounds (VIII) and (IX), which no longer contain the γ-lactone substituent, is in good agreement with the predicted value of 237 mμ, thus supporting the deductions concerning the *K*-band in β-picrotoxinone.



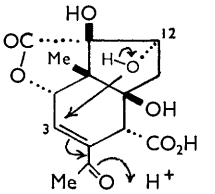
(VIII) R = H
(IX) R = Me



(X) X = :CH_2
(XI) X = :O



(XII) X = :CH_2
(XIII) X = :O



(XIV)

Final proof of structure (V) for β-picrotoxinone has been obtained as follows: Ozonolysis of apopicrotoxinic dilactone, of known structure (X),[10] gave a saturated nor-ketone, apopicrotoxinonic dilactone (XI), which was characterised as the semicarbazone. This nor-dilactone (XI) with acetic anhydride and pyridine gave the monoacetate of an αβ-unsaturated ketone, identical with compound (VI) obtained by acetylation of β-picrotoxinone. Since it would be expected that the β-hydroxy-ketone system in (XI) would be dehydrated under the conditions of the acetylation, the formation of (VI) from (IX) establishes the structural relation between the β-picrotoxinone and apopicrotoxinic dilactone series of compounds.

It was shown previously that ozonolysis of picrotoxic acid gave picrotoxinonic acid which was also obtained by treatment of either α- or β-picrotoxinone with boiling dilute sulphuric acid.[3] On the basis of the established structure (XII) for picrotoxic acid [15,16] picrotoxinonic acid has structure (XIII). The formation of this compound from α-picrotoxinone is undoubtedly mechanistically similar to the formation of picrotoxic acid from picrotoxinin which has been discussed in earlier publications.[15,16] The conversion of β-picrotoxinone into picrotoxinonic acid under acidic conditions can be assumed to involve opening of the δ-lactone ring followed by acid-catalysed Michael addition of the 12-oxygen atom to $C_{(3)}$ of the αβ-unsaturated ketone, as shown in (XIV).

[13] Fieser and Fieser, " Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, 1949, p. 190.
[14] Dorfman, *Chem. Rev.*, 1953, **53**, 1047.
[15] Conroy, *J. Amer. Chem. Soc.*, 1957, 5550.
[16] Burkhill, Holker, Robertson, and Taylor, *J.*, 1957, 4945.

Experimental

Ultraviolet absorption spectra were measured for 95% alcohol solutions with a Unicam S.P. 500 spectrophotometer, and infrared spectra for mineral oil dispersions with a Perkin–Elmer model 21 instrument.

α-*Picrotoxinone* (II).—The ozonide was prepared from picrotoxinin (5 g.) in ethyl acetate (200 ml.) at −70°, isolated by distillation of the solvent *in vacuo*, and decomposed by water (200 ml.) for 15 hr. at room temperature. The crude product was isolated by filtration of the resultant suspension, and purified from warm methyl acetate–light petroleum (b. p. 40—60°), giving needles (2·8 g.) of α-picrotoxinone, m. p. 179—184° (decomp.: rapid heating), $\lambda_{max.}$ 275 mμ (log ε 1·21) with an inflection at 222 mμ (log ε *ca.* 2·25) (Found: C, 57·5; H, 5·0. Calc. for $C_{14}H_{14}O_7$: C, 57·2; H, 4·8%). The 2,4-*dinitrophenylhydrazone* separated from methanol in yellow needles, m. p. 258—268° (decomp.) (Found: C, 50·9; H, 3·7; N, 11·7. $C_{20}H_{18}O_{10}N_4$ requires C, 50·6; H, 3·8; N, 11·8%). Treatment of α-picrotoxinone with sodium hypoiodite and sodium hydroxide gave iodoform, m. p. and mixed m. p. 119°.

β-*Picrotoxinone* (V).—α-Picrotoxinone (2 g.) in methanol (60 ml.) was treated with sodium methoxide (1 drop of a 4% solution of sodium in methanol) and after 15 hr. at room temperature acetic acid (1 drop) was added, the solvent distilled *in vacuo*, and the residue crystallised from ethyl acetate–methanol, giving prisms (1·5 g.) of β-picrotoxinone, m. p. 254° (decomp.) undepressed on admixture with a sample obtained by heating α-picrotoxinone at 195°/1 mm. for 15 min.[3] (Found: C, 57·0; H, 4·9%). Prepared from either α- or β-picrotoxinone with semicarbazide hydrochloride and sodium acetate, the semicarbazone separated from methanol in needles, m. p. 300° (decomp. with prior sintering and decomp. from 210°), $\lambda_{max.}$ 274 mμ (log ε 4·36), $\nu_{max.}$ 3385, 3310, 3120 (OH and >NH), 1760 (γ-lactone), 1740 (δ-lactone), 1680 (amide) and 1657 cm.$^{-1}$ (C=N), (Found: C, 49·2; H, 5·4; N, 11·1. Calc. for $C_{15}H_{17}O_7N_3,H_2O$: C, 48·8; H, 5·2; N, 11·4%). Prepared from β-picrotoxinone with acetic anhydride and pyridine at room temperature for 2 days the *acetate* (VI) separated from acetic acid in needles, m. p. 277° (decomp.), $\lambda_{max.}$ 219 and 318 mμ (log ε 3·88 and 1·52), $\nu_{max.}$ 3509 (OH), 1792 (γ-lactone), 1748 (δ-lactone and *O*-acetate) and 1681 cm.$^{-1}$ (αβ-unsaturated ketone) (Found: C, 57·1; H, 5·0; Ac, 13·3. $C_{16}H_{16}O_8$ requires C, 57·1; H, 4·8; Ac, 12·8%).

α-*Picrotoxinone Acetate* (IV).—Prepared by ozonolysis of picrotoxinin acetate (1 g.) by Hathway's method,[8] α-picrotoxinone acetate separated from ethanol in rhombs (0·75 g.), m. p. 212—215°, $\nu_{max.}$ 1808, 1795 (γ-lactone), 1742 (*O*-acetate), and 1718 cm.$^{-1}$ (saturated ketone) (Found: C, 57·0; H, 4·8. Calc. for $C_{16}H_{16}O_8$: C, 57·1; H, 4·8%).

β-*Picrotoxinone Acetate* (VII).—α-Picrotoxinone acetate (0·9 g.) was suspended in methanol (50 ml.) and treated with sodium methoxide (3 drops of a 4% solution of sodium in methanol) for 2 days at room temperature. After addition of acetic acid (2 drops) the suspension was concentrated (to 7 ml.), and the resultant crystalline precipitate (0·75 g.) recrystallised from acetic acid, giving β-picrotoxinone acetate (VII) (0·5 g.), m. p. 274—276° (decomp.) depressed to 235—244° (decomp.) on admixture with acetate (VI), $\lambda_{max.}$ 223 and 314 mμ (log ε 3·89 and 1·50), $\nu_{max.}$ 3378 (OH), 1799 (γ-lactone), 1736 (δ-lactone and *O*-acetate), and 1678 cm.$^{-1}$ (αβ-unsaturated ketone) (Found: C, 57·0; H, 4·9; Ac, 12·8. Calc. for $C_{16}H_{16}O_8$: C, 57·1; H, 4·8; Ac, 12·8%). This compound was identical with samples obtained (*a*) by Hathway [8] after concentration of the mother-liquors from the crystallisation of α-picrotoxinone acetate and (*b*) by heating the latter compound at 220°/0·1 mm. for 10 min.

Hydrogenolysis of β-*Picrotoxinone*.—Hydrogenolysis of β-picrotoxinone (3 g.) in methanol (100 ml.) with hydrogen (1 mol. absorbed) at atmospheric pressure and Adams catalyst (100 mg.) was complete in 1 hr. The filtered solution was evaporated to dryness *in vacuo*; the residue crystallised from methanol–ethyl acetate as prisms (2·5 g.) of the *acid* (VIII), m. p. 209—212° (decomp.), $\lambda_{max.}$ 233 and 306 mμ (log ε 3·95 and 1·71), $\nu_{max.}$ 3367, 3215 (OH), 1745 (δ-lactone), 1733 (CO_2H) and 1658 cm.$^{-1}$ (αβ-unsaturated ketone) (Found: C, 56·5; H, 5·6. $C_{14}H_{16}O_7$ requires C, 56·8; H, 5·4%). Prepared with diazomethane in ether the *methyl ester* (IX) separated from ethyl acetate–light petroleum (b. p. 60—80°) in needles, m. p. 188—189°, $\lambda_{max.}$ 233 and 302 mμ (log ε 3·98 and 1·71), $\nu_{max.}$ 3472 (OH), 1742 (δ-lactone), 1735 sh (CO_2Me), and 1669 cm.$^{-1}$ (αβ-unsaturated ketone) (Found: C, 57·8; H, 5·9; OMe, 10·0. $C_{15}H_{18}O_7$ requires C, 58·1; H, 5·9; OMe, 10·0%). Prepared with semicarbazide hydrochloride and sodium acetate the *ester semicarbazone* separated from methanol in needles, m. p. 235—240° (decomp.), $\lambda_{max.}$ 265 mμ

(log ε 4·40) (Found: C, 50·0; H, 6·3; N, 11·0; OMe, 8·3. $C_{16}H_{21}O_7N_3,H_2O$ requires C, 49·9; H, 6·0; N, 10·9; OMe, 8·1%).

Apopicrotoxinonic Dilactone (XI).—Ozonised oxygen was conducted through an ethyl acetate solution (400 ml.) of apopicrotoxinic dilactone (1·2 g.) (prepared by Conroy's method [10]) at −70° until the solution had a permanent blue colour (1 hr.). Oxygen was passed through the solution until the blue colour faded and, after warming to room temperature, the resultant solution was hydrogenated (1 mol. absorbed) at atmospheric pressure with 5% palladium–charcoal (0·5 g.). Crystals which separated during the hydrogenation were collected with the catalyst and removed by repeated extraction with boiling methanol. The combined ethyl acetate and methanol solutions were evaporated and the residue crystallised from alcohol, giving rectangular prisms (1·05 g.) of *apopicrotoxinonic dilactone*, m. p. 270° (decomp.) with sintering from 250°, $\nu_{max.}$ 3521, 3425 (OH), 1776 (γ-lactone), 1737 (δ-lactone), and 1718 cm.$^{-1}$ (ketonic C=O) (Found: C, 53·5; H, 5·1. $C_{14}H_{16}O_8$ requires C, 53·8; H, 5·2%). Prepared in the usual way the *semicarbazone* separated from methanol in needles, decomp. 285—300° without melting, $\lambda_{max.}$ 227 mμ (log ε 4·12) (Found: C, 48·8; H, 5·3. $C_{15}H_{19}O_8N_3$ requires C, 48·8; H, 5·2%). Treatment of apopicrotoxinonic dilactone (0·5 g.) with acetic anhydride (5 ml.) and pyridine (2·5 ml.) for 4 days at room temperature, and isolation of the product in the usual way, gave a compound which separated from acetic acid in needles, m. p. 277—278° (decomp.) (Found: C, 57·1; H, 4·8%), and which was shown by mixed m. p. and ultraviolet and infrared spectra to be identical with the acetylation product (VI) from β-picrotoxinone.

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