185. *Picrotoxin and Tutin. Part IV.* The Reducing Properties and Functional Groups.*

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The paper is mainly concerned with an analysis of commonly accepted views of the nature of the picrotoxin series of compounds.

The evidence for the existence of a reducing system in the structures of picrotoxinin and related compounds is examined. It is concluded that, contrary to general opinion, these substances are not themselves reducing compounds but behave as such only if they are degraded to simpler, reducing compounds by the alkaline reagents used in the conventional tests.

The statement that picrotoxinin and picrotin are dilactones is shown to be based on evidence of doubtful validity. Other evidence bearing on this problem is discussed and experiments are described which lead unequivocally to the conclusion that there are two potential centres of acidity in these substances. Their formulation as dilactones is correct if potential acidity in this series is due solely to lactone groups.

The action of alkali on α-bromotutin yields an isomer and the same substance is obtained with diazomethane. The significance of this result is discussed in connection with the relationship of picrotoxinin to α-picrotoxinic acid.

The reaction of dihydropicrotoxinin with acetic anhydride in the presence of a trace of anhydrous ferric chloride gives a diacetyl derivative corresponding to the acetylation of a dihydric alcohol, rather than of a monohydroxy-ether.

Infra-red data are recorded and discussed.

Despite several important recent papers, much of the chemistry of picrotoxin and related substances is still obscure. Our own investigations are not yet complete but in view of two brief reports by Conroy (*J. Amer. Chem. Soc.*, 1951, **73**, 1889), which define the carbon skeleton of picrotoxinin and offer structural formulæ for this compound and its hydration products, we submit some account of our work.

The generally accepted view of the nature of picrotoxinin, $C_{15}H_{16}O_6$, and picrotin, $C_{15}H_{18}O_7$, is that they are dilactones with pronounced reducing properties. Meyer and Bruger (*Ber.*, 1898, **31**, 2958) state that an aqueous solution of picrotoxinin reduces ammoniacal silver nitrate in the cold and Fehling's solution on warming, but that picrotin does not reduce either reagent unless heated to about 70°. This statement is repeated by Horrmann (*ibid.*, 1910, **43**, 1903), and Mercer and Robertson (*J.*, 1936, 288) lay some stress on the strong reducing properties of dihydropicrotoxinin and dihydro*neo*picrotoxinin ("β-dihydropicrotoxinin"), as well as of picrotoxinin itself, in alkaline media. Similarly, α-picrotoxinic acid is credited with strong reducing properties (Meyer and Bruger, *loc. cit.*; Horrmann, *Ber.*, 1913, **46**, 2793; Sutter and Schlittler, *Helv. Chim. Acta*, 1950, **33**, 902). We have frequently observed in examining substances in this series for reducing properties that they are relatively stable to ammoniacal silver nitrate in the cold and, even on heating, reduction often does not take place readily. With ammoniacal silver nitrate sensitised by the addition of a little sodium hydroxide, reduction still does not in general take place in the cold, but does so extremely readily on heating. The fact being taken into account that heating of such substances as picrotoxinin, dihydropicrotoxinin, α-picrotoxinic acid, and dihydro-α-picrotoxinic acid with alkali (even aqueous sodium carbonate) causes profound decomposition to give reducing substances (Sutter and Schlittler, *Helv. Chim. Acta*, 1949, **32**, 1855, 1864; *loc. cit.*), it is clear not only that any reducing properties of picrotoxin and related substances have been exaggerated, but also that these properties may be due to the products of alkaline degradation rather than to the substances themselves. If this were correct there should be a correlation between stability towards alkali and reducing properties. This is indeed so. Of those derivatives of picrotin and picro-

* Part III, *J.*, 1949, 806.

toxinin which may be regarded as derived directly from them, the alkali-stable ones are α-picrototinic acid, picrotoxic acid, the bromopicrotoxinic acids, and Sutter and Schlittler's "Compound C" (*loc. cit.*, 1950). α-Picrotinic acid is non-reducing (Horrmann, *Ber.*, 1912, **45**, 3080); we have likewise found β-bromopicrotoxinic acid and "Compound C" to be non-reducing. Picrotoxic acid is described by Horrmann (*Annalen*, 1916, **411**, 273) as non-reducing but Harland and Robertson (*J.*, 1939, 937) refer to its strong reducing properties. We have found crude preparations of picrotoxic acid to show some reducing effect, due probably to unchanged picrotoxinin, but this effect is absent in well-purified specimens. Tables 1 and 2 record the behaviour of a range of substances in reduction

TABLE 1.

Compound	NH_3–$AgNO_3$	Sensitised NH_3–$AgNO_3$	Na_2CO_3
Ozonised *neo*picrotoxinin [1]	+		1
Glucose	+		3
*neo*Picrotoxinin	+		4
Ozonised picrotoxinin [2]	+		
*neo*Tutin [3]	+ (very slight)		5 [4]
Picrotoxin	+		4
Tutin	+ (slight)		6
α-Picrotoxinic acid	+ (very slight)		2
Cane sugar (comml.)	+ (trace)	1	
Acetyl*neo*picrotoxinin	+ (trace)	2	4
β-Bromopicrotoxinic acid	—	—	
"Compound C"	—	—	
Picrotoxic acid	—	—	
Methyl α-picrotinate	—	—	
Methyl β-picrotinate	—	—	

[1] This was the only substance tested which gave a positive result in the cold. [2] A specimen two years old. [3] Unpublished work. [4] Orange-red colour.

tests. Column 2 in each table gives the results of two sets of experiments in each of which the individual tests were carried out simultaneously, and the order of listing is that in which reduction of ammoniacal silver nitrate was first observed. Unless otherwise indicated a positive result implies marked reduction. It may be noted that if such substances as picrotoxinin, dihydropicrotoxinin, and picrotin are heated rapidly with an

TABLE 2.

Compound	NH_3–$AgNO_3$	Sensitised NH_3–$AgNO_3$	Na_2CO_3
Dihydro*neo*picrotoxinin	+		7
Dihydropicrotoxinin	+ (slight)		5
Bromo*neo*picrotoxinic acid	+ (trace)	2	3
Dihydro-α-picrotoxinic acid	+ (trace)	1	1
Picrotin	+ (slight)		2
Acetylpicrotin	+ (very slight)		6
α-Dihydrotutin	+ (slight)		4

ammoniacal silver nitrate solution which contains the smallest possible excess of ammonia they may be boiled for short periods without any reduction being observed. Col. 3 in each table lists the behaviour towards sensitised ammoniacal silver nitrate of those substances showing a negative or very slight positive effect in the first series of experiments. Each test was performed separately and the order of listing is an approximate indication of the extent of reduction.

The correlation between the existence of a group of alkali-stable compounds and their non-reducing properties is evident. An attempt was now made to gauge whether, in a group of alkali-unstable compounds, there is any correlation between the ease of degradation and the reducing power. Some indication is given by the observation that, although dihydropicrotoxinin is completely degraded by hot aqueous sodium carbonate, yet dihydrotutin under comparable conditions is only partly degraded (unpublished work). The latter (see Table 2) has but little effect on ammoniacal silver nitrate, but the former gives rapid evidence of some reduction. Again, although no difficulty has been experienced in converting bromopicrotoxinin into bromopicrotoxinic acid by the action of alkali, yet the corresponding reaction between bromo*neo*picrotoxinin and alkali (Slater, *J.*, 1949, 806)

must be conducted carefully to avoid alkaline degradation. It is noteworthy that, whereas β-bromopicrotoxinic acid appears in Table 1 as a non-reducing substance, yet bromo*neo*-picrotoxinic acid shows slight reducing properties. As a useful guide to the extent of degradation caused by alkali, one may take the development of the characteristic yellow colour which appears during the reaction. This is due to the formation of some as yet unisolated substance which is an indicator, being colourless in acid solution and yellow in alkali. The last columns in Tables 1 and 2 show the order in which the substances listed developed a certain arbitrary depth of yellow colour when heated with 2N-sodium carbonate at 100°, the tests being performed simultaneously. There is reasonable correlation between these columns and the second columns, if it is borne in mind that solubility factors probably affect the orders somewhat (*e.g.*, dihydropicrotoxinin and dihydro*neo*picrotoxinin dissolved very slowly in the carbonate test). The behaviour of α-picrotoxinic acid and dihydro-α-picrotoxinic acid is anomalous in that attack by carbonate is rapid whereas their reducing power is slight.

From the above results we conclude that the evidence for the existence of a reducing system in the structures of compounds of this series is better interpreted by the theory that any observed reduction is dependent on a preliminary degradation to simpler, reducing substances under the alkaline conditions of the tests used.

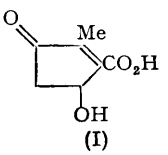
The evidence cited for the presence of two lactone groups in picrotoxinin and picrotin is most unconvincing. It was early observed (Paternò and Oglialoro-Todaro, *Gazzetta*, 1876, **6**, 531, and succeeding papers; Meyer and Bruger, *loc. cit.*, 1898) that picrotin, although unaffected by cold dilute aqueous sodium carbonate, dissolves in cold dilute aqueous sodium hydroxide and is recovered unchanged on acidification of this solution. This behaviour is typical of lactones (although not exclusively so). Under more drastic conditions picrotin, $C_{15}H_{18}O_7$, is converted irreversibly into the monobasic β-picrotinic acid, $C_{15}H_{20}O_8$, and finally into the dibasic picrotindicarboxylic acid, $C_{15}H_{22}O_9$ (Horrmann, *loc. cit.*, 1916). This addition of the elements of water is accompanied by the formation of new hydroxyl groups as revealed by Zerewitinoff determinations. Similarly, picrotoxinin, $C_{15}H_{16}O_6$, may be converted irreversibly into the monobasic α-picrotoxinic acid, $C_{15}H_{18}O_7$, and the dibasic picrotoxinindicarboxylic acid, $C_{15}H_{20}O_8$. On these ground picrotin and picrotoxinin have been regarded as dilactones. Unfortunately, as was clearly recognised by Horrmann (*loc. cit.*, 1916), the connection between the parent substances and their products of hydration is quite obscure. It has never been possible to isolate the true hydroxy-acids corresponding to picrotin and picrotoxinin. The only relationship of this sort yet established is between bromo*neo*picrotoxinin and the monobasic acid obtained by treating this substance with alkali (Slater, *loc. cit.*, 1949). Here we have the scheme :

$$\underset{C_{15}H_{16}O_6}{\text{Picrotoxinin}} \xrightarrow{H_2\text{–Pd–H}\cdot} \underset{C_{15}H_{16}O_6}{\textit{neo}\text{Picrotoxinin}} \underset{Zn}{\overset{Br_2}{\rightleftharpoons}} \underset{C_{15}H_{15}O_6Br\ (\text{lactone})}{\text{Bromo}\textit{neo}\text{picrotoxinin}} \underset{\text{Heat}}{\overset{OH'}{\rightleftharpoons}} \underset{C_{15}H_{17}O_7Br\ (\text{hydroxy-acid})}{\text{Bromo}\textit{neo}\text{picrotoxinic acid}}$$

We may state with certainty, therefore, that *neo*picrotoxinin (and hence presumably picrotoxinin) contains one lactone group. The close relationship between these two substances and picrotin (Slater and Wilson, *Nature*, 1951, **167**, 324) makes it equally probably that this also contains a lactone group.

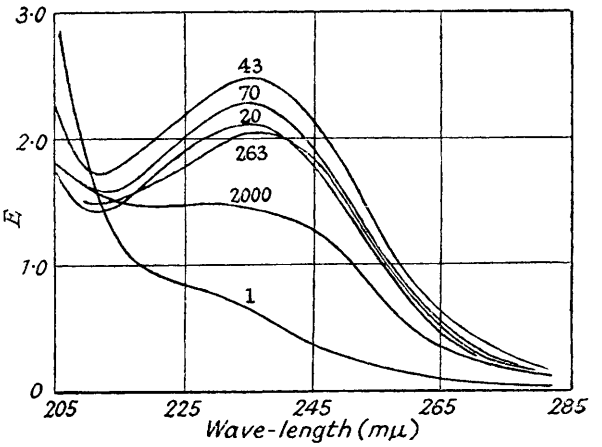
The question of the existence or otherwise of a second lactone group is much more difficult to answer. The recent demonstration by Sutter and Schlittler (*loc. cit.*, 1949) that picrotoxinin is extensively degraded by heating with aqueous sodium carbonate, and the application of this method to the splitting of dihydro*neo*picrotoxinin and picrotin (Slater and Wilson, *loc. cit.*), render suspect arguments based upon transformations under any but the mildest alkaline conditions. If we consider, for example, Horrmann's preparation of picrotindicarboxylic acid, in which picrotin was kept at room temperature with aqueous barium hydroxide for 3 days and then heated, it is plausible to suppose that under these conditions picrotindicarboxylic acid may well arise by profound internal modification of picrotin, or even complete degradation followed by interaction of the intermediate products formed. The carbonate degradation products of picrotin include the acid (I) which shows a characteristic absorption maximum in the ultra-violet at

235 mμ. With this as a guide to the progress of any alkaline degradation, picrotin was kept at room temperature with aqueous barium hydroxide and samples of the reaction mixture were removed from time to time. The absorption spectra of these samples are recorded in Fig. 1. The steady waxing and waning of the 235-mμ maximum is most striking and it is reasonable to attribute this to the formation and decay of an intermediate which contains a chromophore similar to that of (I). This intermediate reaches its maximum concentration after 2—3 days and is then consumed in some subsequent reaction. The amount of this compound formed is difficult to estimate accurately, as a knowledge of the absorption of other substances present is required. However, even a conservative estimate shows that a large proportion of picrotin has passed through the cycle. This could not be a subsidiary



(I)

FIG. 1.



Absorption curves of 1-*ml. aliquots* (*diluted to* 25 *ml. with dilute hydrochloric acid*) *removed after* x *hours from a reaction mixture containing picrotin* (0·5 *g.*) *in aqueous barium hydroxide* (0·75 *g. of barium hydroxide in* 15 *ml. of water*). *Measurements in cell of* d = 1 *cm.*

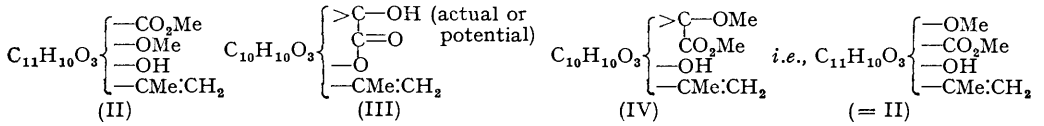
reaction in the light of the extremely high yield (96%) of picrotindicarboxylic acid obtained by Horrmann. If these views are correct it is no longer permissible to assume a simple relationship between picrotin and picrotindicarboxylic acid, and the chief cited evidence for the existence of a second lactone group in picrotin is invalidated.

If picrotindicarboxylic acid is not a simple derivative of picrotin, then the same may well be true of β-picrotinic acid. On the other hand, α-picrotinic acid could not be formed by the above mechanism, for it has been prepared in strongly oxidising solution (Horrmann, *Ber.*, 1912, **45**, 3434) which would be expected to destroy the intermediate. It can also be obtained from picrotin by acid hydrolysis (*idem*, *loc. cit.*, 1912). There is little experimental evidence on the relation of picrotoxinindicarboxylic acid to picrotoxinin.

The above discussion is intended to direct attention to the weakness of the argument on which presumably picrotin and picrotoxinin are formulated as dilactones. That alternative formulations are possible has, indeed, been pointed out (Harland and Robertson, *loc. cit.*, 1939). There is, however, other available evidence which does not yet appear to have been examined. The conversion of picrotin into α-picrotonic acid, $C_{15}H_{20}O_8$, and of picrotoxinin into picrotoxic acid, $C_{15}H_{18}O_7$, takes place under the influence of either acids or alkoxides (Horrmann, *locc. cit.*, 1912, 1916). There is evidence that these substances still behave towards alkali as lactones and, if it can be assumed that the newly-formed carboxyl and hydroxyl groups of these monobasic acids have come from a lactone group (their formation being accompanied by some other change as yet not understood), the parent substances would then be dilactones. Horrmann (*loc. cit.*, 1916) has stated that picrotoxic acid is resistant to attack by alkali and cannot therefore contain a lactone group. The position is actually the reverse, as was clear from this author's description of

the preparation of the methyl ester of picrotoxic acid, which was precipitated by acid from alkaline solution. This substance, although insoluble in cold dilute aqueous sodium carbonate, is readily soluble in cold dilute aqueous sodium hydroxide and is reprecipitated unchanged on acidification. Similarly, in the preparation of the methyl ester of α-picrotonic acid, the alkaline reaction mixture is acidified, whereupon the ester separates. Its behaviour towards alkali we have likewise found to be typically that of a lactone.

A third line of evidence can be followed in Sutter and Schlittler's recent paper (*loc. cit.*, 1950) dealing with the action of diazomethane on some substances in this series. It is known that diazomethane may attack lactones with formation of the methyl esters of the corresponding hydroxy-acids (Fischer and Hofmann, *Z. physiol. Chem.*, 1937, **245**, 139). In such reactions fundamental molecular rearrangements are unlikely to occur and it is interesting therefore that bromopicrotoxinin reacted with diazomethane in the presence of methyl alcohol to give the methyl ester of bromopicrotoxinic acid, presumably by attack of a lactone ring, and that under similar conditions α-picrotoxinic acid, the product of debromination of bromopicrotoxinic acid, gives the methyl ester of picrotoxinindicarboxylic acid, indicating the presence of a second lactone group. Inasmuch, however, as the relationship of α-picrotoxinic acid to picrotoxinin is not simply that of hydroxy-acid to lactone, this series of reactions we regard as of less significance than the reaction of picrotoxinin itself with diazomethane, which appears to us to provide the most satisfactory evidence yet published for the presence of two lactone rings in picrotoxinin. The substance produced, Sutter and Schlittler's "Compound C," was formulated by these workers as (II), but because of their difficulty in interpreting the nature of the residue $C_{11}H_{10}O_3$ they regarded Compound C as being the product of an extremely complex reaction. The formation of Compound C and its general behaviour can, however, be explained quite simply if the partial formula (III) is assigned to picrotoxinin and (IV) to Compound C.

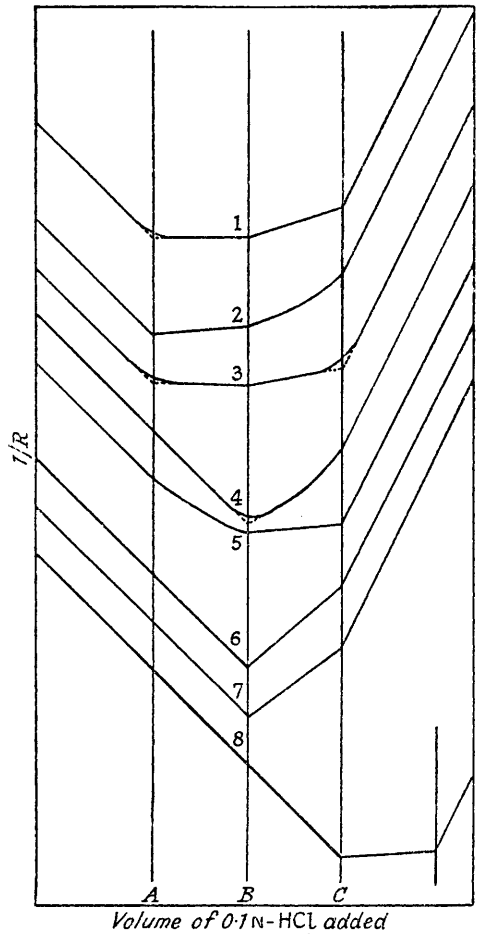


The methylation of alcoholic hydroxyl groups by diazomethane is possible if acidifying groups such as $-CO_2R$ are adjacent (Schmidt *et al.*, reported by Eistert in "Newer Methods of Preparative Organic Chemistry," p. 520; *Ber.*, 1934, **67**, 2120, 2127). If this scheme is correct, any postulated second lactone group must be present in the C_{11} residue, and its existence or otherwise should be clearly evident from the behaviour of Compound C. Although the reaction with cold dilute aqueous sodium hydroxide is sluggish, Compound C does indeed behave as a lactone, dissolving on long shaking and being reprecipitated unchanged on acidification.

As mentioned above, the methyl esters of picrotoxic and α-picrotinic acids are alkali-soluble and it was thought desirable to measure the potential acidity of these and other substances in this series quantitatively. Back-titration using indicators (phenolphthalein) has been used by other workers, and by this method picrotin appears to consume one equivalent of alkali in the cold (Horrmann, *loc. cit.*, 1910). Applied to the methyl ester of α-picrotinic acid, it showed 0·1 equiv. of alkali consumed per mole of ester, suggesting that the indicator used was unsuitable, and a more precise study, based on conductimetric titrations, was therefore undertaken. This yielded results of considerable interest which are summarised in Figs. 2 and 3. The curves of Fig. 2 all refer to substances which are recovered unchanged on acidification of their alkaline solution, and any conclusions drawn from the curves are therefore valid for the parent substances and not subject to any difficulties of interpretation through the occurrence of irreversible changes of an unknown and possibly profound nature. It is quite clear that picrotin and dihydropicrotoxinin contain two potential acidic groups, picrotoxic acid one such group in addition to the carboxyl group, and the methyl ester of α-picrotinic acid one. On the other hand, β-bromopicrotoxinic acid, bromo*neo*picrotoxinic acid, β-picrotinic acid, and dihydro-α-picrotoxinic acid show no acidity other than that due to their carboxyl groups. If potential acidity in this series can be attributed solely to the presence of lactone groups, we have in these

curves the first unequivocal evidence that picrotin and picrotoxinin are dilactones, and that one lactone group remains in their products of acid hydrolysis, α-picrotinic acid and picrotoxic acid, respectively. It may be noted that neither of these two acids has been converted into a stable dicarboxylic acid, and each has become stabilised towards the degradative action of alkali. On the other hand, in β-picrotinic acid and dihydro-α-

FIG. 2.

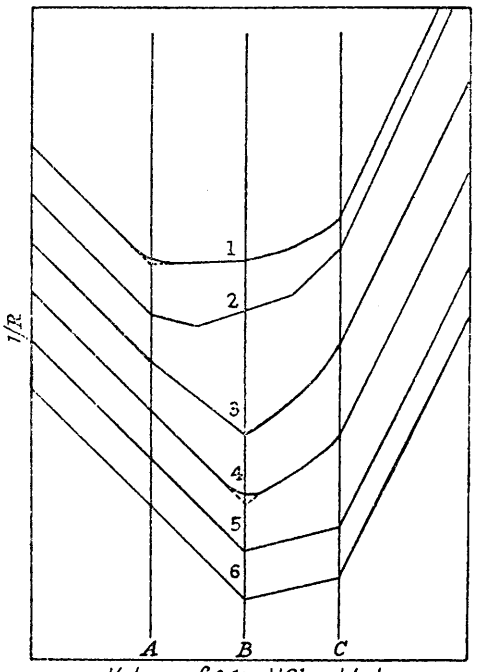


Volume of 0·1 N-HCl added
A-B = B-C = 1 equivalent

1, *Picrotin.*
2, *Dihydropicrotoxinin.*
3, *Picrotoxic acid.*
4, *Dihydro-α-picrotoxinic acid.*
5, *Methyl ester of α-picrotinic acid.*
6, *β-Bromopicrotoxinic acid.*
7, *Bromoneopicrotoxinic acid.*
8, *Potassium salt of β-picrotinic acid.*

Schematic back-titration curves
(*see* p. 1050 *for actual values*).

FIG. 3.



Volume of 0·1 N-HCl added
A-B = B-C = 1 equivalent

1, *Picrotoxinin.*
2, neo*Picrotoxinin.*
3, *Dihydroneopicrotoxinin.*
4, *α-Picrotoxinic acid.*
5, *Tutin.*
6, (α-)*Dihydrotutin.*

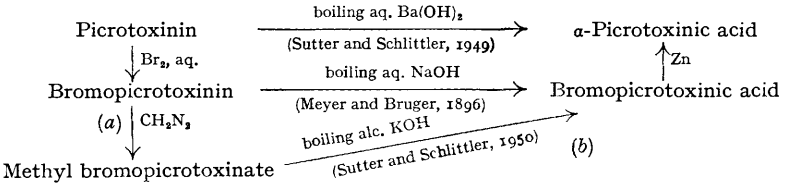
Schematic back-titration curves
(*see* p. 1050 *for actual values*).

picrotoxinic acid one of the lactone groups has been modified so that it is unaffected by cold dilute aqueous sodium hydroxide, although the instability towards more vigorous alkaline treatment is retained.

The uncertain relationship between picrotoxinin and α-picrotoxinic acid has been referred to above. In spite of Sutter and Schlittler's assumption (*loc. cit.*, 1950) that the change from the former to the latter is simply the opening of a lactone ring, the evidence

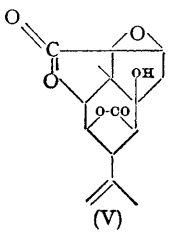
is clearly against this view and the above results serve to emphasize the difference. In particular we emphasize (i) the very different behaviour on titration of picrotoxinin and α-picrotoxinic acid on the one hand, and of dihydropicrotoxinin and dihydropicrotoxinic acid on the other; (ii) the hitherto apparently unrecognised reversible relationship between dihydropicrotoxinin and the dibasic acid (as yet unisolated) into which it is converted by cold dilute alkali; and (iii) the very different behaviour of picrotoxinin and α-picrotoxinic acid towards diazomethane.

The methods by which picrotoxinin has been converted into α-picrotoxinic acid are summed up in the scheme :

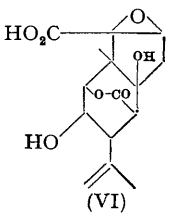


A noteworthy feature of all these methods is that each makes use of boiling alkali at some stage, and the suspicion arises that the rigour of this treatment may cause some irreversible change in addition to the opening of a lactone ring and that possibly these two effects are separated in the steps labelled (*a*) and (*b*). A closer investigation has shown, however, that the irreversible change is brought about under quite mild conditions; for instance, when bromopicrotoxinin is dissolved in dioxan and treated in the cold with a little alkali it is readily converted into bromopicrotoxinic acid; and the hydrolysis of the methyl ester is brought about immediately by suspending it in cold water and adding dilute aqueous sodium hydroxide. Furthermore, the acid formed regenerates the original ester on esterification.

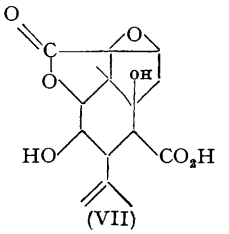
The action of alkali on tutin is of considerable interest. It is clear from Figs. 2 and 3 that in going from picrotoxinin to bromopicrotoxinin there is an effective loss of one of the potentially acidic groupings. Tutin is titrated as a potentially monobasic acid, and it would be expected, following the close analogy so far revealed in the chemistry of tutin and picrotoxinin, that bromotutin would show *no* potential acidity. This is indeed the case. However, dilute aqueous sodium hydroxide is not without action on this substance—a (neutral) isomeride is readily produced and, furthermore, diazomethane has the same effect. It is clear that by a fortunate circumstance the dual action of alkali or diazomethane on bromopicrotoxinin (isomerisation and lactone opening) is modified in the case of bromotutin so that one effect (lactone opening) is eliminated while the other (isomerisation) remains operative. The complexity of the former reaction is thereby revealed.



(V)



(VI)



(VII)

Conroy (*loc. cit.*, 1951) has briefly discussed the relation of picrotoxinin to α-picrotoxinic acid in terms of their infra-red spectra. He also concludes that the acid is not a simple (chemical) hydrate of picrotoxinin, and traces the isomerisation effect to a change from a γ- to a δ-lactone system. The formulæ advanced for picrotoxinin, α-picrotoxinic acid, and picrotoxic acid are (V), (VI), and (VII), respectively.

The absence of any reducing group in these structures is in agreement with our own findings. The presence of two five-membered lactone rings in picrotoxinin is postulated on the basis of the split peak in the carbonyl absorption region of the infra-red spectra with maxima at 5·57 and 5·63 μ. The hydroxyl group gave a farly sharp band at 2·90 μ.

As the purely chemical evidence outlined above had left the problem of the number of lactone groups present dependent on the interpretation of the precise cause of potential acidity in this group, we also studied the infra-red spectra. This was made possible through the courtesy of Professor Roger Adams. The interpretation of the curves is complicated by the differences between the spectra of the solids and their solutions. When a Nujol mull of the solid was used, picrotin and *neo*picrotoxinin showed a split peak in the carbonyl region, whereas picrotoxinin itself showed only broad absorption. In dioxan solution, however, single bands were obtained in each case. For this reason Dr. E. M. Petersen, to whom we are greatly indebted for the infra-red data and their interpretation, concludes that the presence of a dilactone structure is doubtful.* The presence of a single five-membered lactone ring in each of the substances examined was inferred. The presence of the other functional groups, as deduced from the chemical evidence, is in accordance with the spectral characteristics.

Conroy's formulæ serve as a useful basis for further discussion, the complete definition of the carbon skeleton in particular being a major advance. Further experimental support appears necessary, and will no doubt be forthcoming, before the validity of the finer details of structure can be assessed. Attention may be drawn, however, to the over-simplification of the chemistry of picrotoxic acid implied by (VII). In particular: (i) The sole structural difference between picrotoxinin (V) and picrotoxic acid (VII) is attributed to the opening of a lactone ring: the very different behaviour of the two substances towards dilute alkali (see Figs. 2 and 3) implies some other structural difference. (ii) Picrotoxic acid is not attacked by alkali under conditions which bring about the fission of picrotoxinin: (VII) would be expected to undergo the Schlittler degradation. (iii) The characteristic bromination reaction of picrotoxinin and α-picrotoxinic acid might reasonably be expected to take place with (VII), but picrotoxic acid does not behave in this way.

Of the postulated functional groups there remains for discussion the ether linkage, conventionally assumed to be present to account for the inert oxygen atom of picrotoxinin and picrotin of otherwise undefined function. Here again positive evidence is lacking and it may be noted that no ether linkage has been found in any degradation product of established structure. Conroy's formulation assumes the presence of an ethylene oxide, which should presumably be capable of conversion into the corresponding glycol or a derivative. The simplest type of hydration is that usually brought about by the action of water at high temperatures. We have found, however, that picrotoxinin and dihydropicrotoxinin are recovered substantially unchanged after being heated with water at 150° (2 hours), and even at 200° (1 hour); although there is much obvious thermal decomposition, dihydropicrotoxinin is partly recovered. Another characteristic reaction of ethylene oxides is their conversion into the corresponding glycol diacetate under the influence of acetic anhydride and a trace of anhydrous ferric chloride (Knoevenagel, *Annalen*, 1914, **402**, 135). As applied to dihydropicrotoxinin, this reaction gave a quantitative yield of a diacetate, $C_{19}H_{22}O_8$, differing however by the elements of water from that expected if an oxide link had been attacked in the normal way, *viz.*, $C_{19}H_{24}O_9$. A dehydration accompanying the opening of the oxide link is unlikely since the substance gives no signs of unsaturation. Its formation, in fact, is most simply regarded as the acetylation of a dihydroxy-compound, and on this view dihydropicrotoxinin is a dihydric alcohol rather than a monohydroxy-ether. The substance is non-reducing and does not give a yellow colour when warmed with aqueous sodium carbonate.

The reaction of picrotoxinin itself and acetic anhydride in the presence of ferric chloride

* (Supplementary report by Dr. E. M. Petersen on the infra-red spectrum of a new specimen of picrotoxinin. Received, 28.1.52.) The infra-red spectrum in the 6 μ region of the new sample of picrotoxinin seems to have two bands in "Nujol" suspension and in solution instead of the one broad band which I observed before. The rest of the spectra are the same. The bands in the "Nujol" mull occur at 1787 and 1765 cm.$^{-1}$ (5·60 and 5·67 μ); they are strong and well resolved. In dioxan solution there is a strong band at 1802 cm.$^{-1}$ (5·55 μ) and a second band at 1793 cm.$^{-1}$ (5·58 μ). The latter band is not resolved into a sharp peak but is more of a shoulder on the 1802-cm.$^{-1}$ band. These different results may indicate that the new sample is purer than the original or that the sample preparation scattered more light in the first case, thus obscuring the band structure. In view of these new results and the chemical evidence there appear to be two lactone groups in picrotoxinin.

proceeded differently—on long storage in the cold a deep violet-purple colour slowly developed, suggesting the formation of an enol, but no pure material could be isolated from the product obtained by decomposing the reaction mixture with water.

Experimental

Reduction Tests.—A bulk sample of ammoniacal silver nitrate was prepared by adding aqueous ammonia (*d* 0·88) dropwise to 0·1N-aqueous silver nitrate until the precipitate dissolved and a slight excess of ammonia was present. The reagent was stable to heat. In the experiments summarised in the second columns of Tables 1 and 2 approximately equal small samples of the substances listed were heated with the ammoniacal silver nitrate in a water-bath (all tests in each group being done simultaneously), the time of heating being approximately 5 minutes to reach the boiling point of the bath which was then kept boiling for about 10 minutes. In the tests summarised in cols. 3 of Tables 1 and 2, each of which was carried out separately, a little of the bulk ammoniacal silver nitrate, containing a sufficient excess of ammonia to give reasonable stability to heat in the final reagent, was sensitised by adding a drop of aqueous sodium hydroxide prepared from AnalaR alkali. There sulting clear solution was stable to the conditions of heating used in the tests. Each test was performed by adding a small amount of substance, heating the mixture rapidly nearly to the boiling point, and continuing at this temperature for about 30 seconds.

Conductimetric Titrations.—A known weight of each substance was dissolved in a measured volume (excess) of 0·1N-sodium hydroxide, and the solution titrated in a semimicro-conductimetric cell by measuring the resistance. A continuous stream of previously purified air saturated with water vapour was bubbled through the solution to ensure effective stirring. The curves of Figs. 2 and 3 have been proportionately adjusted so that for all titrations the distances *AB* and *BC*, each representing one (theoretical) equivalent of alkali consumed by the substance titrated, are the same. The experimental values are given below. The molar concentrations of the solutions titrated varied slightly and any comparison of the relative slopes of the curves is subject to a corresponding small error. Although α-picrotoxinic acid is included in Fig. 3, it has on one occasion been recovered after acidification of the titration solution. Its stability towards alkali, however, is not so well marked as that of the dihydro-acid. Similarly, picrotoxinin has been recovered unchanged from solution in an excess of dilute alkali, although not under the controlled experimental conditions of the recorded back-titrations. Dihydropicrotoxinin, however, is reasonably stable to cold dilute alkali and is always recovered on acidification. The curve for *neo*picrotoxinin is difficult to interpret. It is reproducible.

Data for Figs. 2 and 3.

Substance (Fig. 2)	Wt., mg.	G.-mols. × 10^4	NaOH consumed, g.-equiv. × 10^4 A–B	B–C	Total	Number of actual or potential acidic groups
Picrotin	46·5	1·50	1·36	1·60	2·96	2
Dihydropicrotoxinin	36·8	1·25	1·30	1·20	2·50	2
Picrotoxic acid	43·2	1·39	1·30	1·40	2·70	2
Dihydro-α-picrotoxinic acid	30·1	0·964	0	0·93	0·93	1
Methyl α-picrotinate	41·0	1·20	0	1·23	1·23	1
β-Bromopicrotoxinic acid	22·7	0·584	0	0·55	0·55	1
Bromo*neo*picrotoxinic acid	83·5	2·14	0	2·25	2·25	1
Potassium β-picrotinate	24·3	0·665	0	0	0	0
Fig. 3.						
Picrotoxinin	45·3	1·55	1·55	1·55	3·10	2
*neo*Picrotoxinin	38·5	1·32	—	—	2·60	2
Dihydro*neo*picrotoxinin	25·8	0·878	0	0·85	0·85	1
α-Picrotoxinic acid	29·2	0·943	0	0·90	0·90	1
Tutin	34·6	1·18	0	1·30	1·30	1
(α-)Dihydrotutin	38·3	1·30	0	1·35	1·35	1

Action of Alkali on Bromotutin.—α-Bromotutin was shaken with a known slight excess of 0·1N-sodium hydroxide (calculated on the assumption that α-bromotutin was a potential monobasic acid). After 15 minutes a flocculent precipitate appeared suddenly. The mixture was kept for some hours, and the precipitate then collected and crystallised from methyl alcohol. The *substance* melted at 267° (Found: C, 48·1; H, 4·94; Br, 21·7. $C_{15}H_{17}O_9Br$ requires C, 48·3; H, 4·60; Br, 21·4%). The aqueous mother-liquor from the above preparation on titration showed no loss of alkali.

Action of Diazomethane on Bromotutin.—An excess of an ethereal solution of diazomethane

was added to α-bromotutin (220 mg.), and the mixture kept at room temperature for 60 hours. The resulting solution was evaporated to half volume and set aside. The crystalline material which separated melted at 210°, raised to 268° after three crystallisations from ethyl alcohol. It showed no mixed m. p. depression with the above substance.

Acetylation of Dihydropicrotoxinin.—Dihydropicrotoxinin (0·5 g.) was kept for a week at room temperature with an excess of acetic anhydride containing sufficient anhydrous ferric chloride to give the solution a pale yellow colour. After addition of water, the *diacetate* (0·5 g.) slowly separated and had m. p. 243—244°, raised to 245° by crystallisation from methyl alcohol (Found: C, 60·1, 60·15; H, 5·87, 5·85; Ac, 21·3. $C_{19}H_{22}O_8$ requires C, 60·31; H, 5·82; 2 Ac, 22·8%).

REPORT ON THE INFRA-RED SPECTRA OF PICROTOXININ, PICROTIN, AND *neo*PICROTOXININ.

By E. M. PETERSEN (University of Illinois).

Picrotoxinin.—The spectrum shows an unconjugated C=C bond at 1649 cm.$^{-1}$ (6·06 μ). In the solid state the C=O absorption is broad and not well resolved, ranging from 1790 to 1766 cm.$^{-1}$ (5·59—5·66 μ); however, in dioxan solution the C=O linkage appears as a single band at 1799 cm.$^{-1}$ (5·58 μ). This result would indicate one lactone system. A strong free hydroxyl absorption appears at 3464 cm.$^{-1}$ (2·89 μ) in the solid, and at 3410 cm.$^{-1}$ (2·93 μ) in the dioxan solution. The spectrum shows very strong absorption at 1163 cm.$^{-1}$ (8·60 μ) which is in the range of the C–OH vibrations of tertiary alcohols.

Picrotin.—The spectrum shows no C=C absorption. In the Nujol mull two bands appear at 1803 and 1773 cm.$^{-1}$ (5·55 and 5·64 μ). The 1773 band is stronger. However, in solution, one band appears at 1799 cm.$^{-1}$ (5·58 μ) in dioxan and at 1794 cm.$^{-1}$ (5·57 μ) in nitromethane or methanol. The OH absorption in the Nujol spectrum indicates the possibility of more than one OH since there is a band at 3333 cm.$^{-1}$ (3·00 μ) and a weaker broad band at 3120 cm.$^{-1}$ (3·21 μ). In dioxan solution one broad band appears at 3332 cm.$^{-1}$ (3·00 μ). There is no strong absorption at 1163 cm.$^{-1}$ (8·60 μ) as in picrotoxinin, but a fairly strong band appears at 1158 cm.$^{-1}$ (8·64 μ) which is also in the range of tertiary alcohols. The possibility of a dilactone structure seems rather doubtful since the C=O absorption is not high enough.

neo*Picrotoxinin.*—The spectrum of the solid shows two well-resolved bands at 1803 and 1763 cm.$^{-1}$ (5·55 and 5·67 μ). This would seem to indicate two lactone systems. However, in solution only one C=O absorption appears, again in about the same place as in picrotoxinin and picrotin. No C=C absorption appears. One sharp strong OH absorption band appears at 3497 cm.$^{-1}$ (2·86 μ) in the solid and at 3390 cm.$^{-1}$ (2·95 μ) in the solution. The 3497 cm.$^{-1}$ band is high enough to indicate almost no bonding of the OH.

The shift of the carbonyl bands of these three compounds to approximately the same frequency in solution is difficult to explain. It may possibly be due to a high degree of association in the solid which is not present in solution. The solution spectra would indicate that the lactone systems of the compounds are similar and are most probably five-membered saturated lactones.

Note (by S. N. SLATER).—The absence of C=C absorption in *neo*picrotoxinin may be correlated with its formulation as a tetra-substituted olefin (Slater, *loc. cit.*, 1949), although the work of Slater and Wilson (*loc. cit.*, 1951) shows clearly that the system present must be a simple *iso*propylidene one. Jones *et al.* (*J. Amer. Chem. Soc.*, 1950, **72**, 86) have shown that the band normally observed in the region 1580—1680 cm.$^{-1}$, which is due to the stretching motion of the C=C bond, may be absent, *e.g.*, in unconjugated $\Delta^{8:14}$ and $\Delta^{9:11}$ compounds of the steroid series.

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