62. *Oenanthotoxin and Cicutoxin. Isolation and Structures.*

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Oenanthotoxin, the toxic principle of *Oenanthe crocata,* has been isolated pure and assigned the formula $C_{17}H_{22}O_2$. It is accompanied in the plant by the closely related oenanthetol, $C_{17}H_{22}O$, and oenanthetone, $C_{17}H_{20}O$.

Cicutoxin, the toxic principle of *Cicuta virosa,* has been isolated pure and crystalline for the first time. It is isomeric with oenanthotoxin, and is accompanied in the plant by cicutol, $C_{17}H_{22}O$.

Spectroscopic and degradative evidence defines oenanthotoxin as an all-*trans*-heptadeca-2 : 8 : 10-triene-4 : 6-diyne-1 : 14-diol, and cicutoxin as an all-*trans*-heptadeca-8 : 10 : 12-triene-4 : 6-diyne-1 : 14-diol; the configuration at $C_{(14)}$ in cicutoxin is opposite to that in oenanthotoxin. With a high degree of probability oenanthetol is 14-deoxyoenanthotoxin, oenanthetone is the 14-ketone from 1-deoxyoenanthotoxin, and cicutol is 14-deoxycicutoxin.

Some of the novel features presented by these compounds are mentioned.

THE poisonous character of *Oenanthe crocata* (hemlock water dropwort) and *Cicuta virosa* (cowbane; water hemlock) (Umbelliferae) has been known for many centuries; they are said to have been used for both medicinal and criminal purposes. The most poisonous parts of *Oenanthe crocata,* the roots, resemble a bunch of small white carrots, giving the plant the name "five-finger death"; when a root is broken drops of a pale yellow oil appear and rapidly become brown in the air. This oil, which contains the toxin, is easily extracted with ether. The plant is responsible for much poisoning of stock (Fenton and Robertson, *Bull. Edinb. Coll. Agric.*, 1931, **4**, 13, 42) and cases of poisoning among humans, especially children, are common (Pohl, *Arch. exp. Path. Pharmak.*, 1894, **34**, 265; *Lancet*, 1947, II, 485; 1952, I, 155, 316). The water hemlock, *Cicuta virosa* (for description and illustration see Bull. U.S. Dept. Agric., 1913, No. 69), although indigenous to, is less common in, England than *Oenanthe,* but it has caused numerous fatalities in Europe and the United States amongst humans and animals. The rhizome, a bulbous structure with characteristic air-cells, weighs up to 1 kg. and contains a yellow oil similar to that of *Oenanthe.*

Oenanthotoxin and cicutoxin, the poisonous principles, have a very similar pharmacological action, causing violent convulsions and death. They have long been regarded as closely related to each other and possibly also to the picrotoxin group. They were recognised as neutral, non-nitrogenous compounds, but until recently little progress was made in their chemical investigation since neither could be obtained pure or crystalline. Pohl (*loc. cit.*) examined and compared them, and gave crude oenanthotoxin the formula $C_{17}H_{22}O_5$; his crude cicutoxin preparations were somewhat richer in carbon. Jacobson (*J. Amer. Chem. Soc.*, 1915, **37**, 916) gave crude cicutoxin the formula $C_{19}H_{26}O_3$; it resinified in air, reacted vigorously with bromine, and gave oxalic acid on oxidation. Jacobson suggested a structure for cicutoxin containing a *cyclo*pentane and dihydropyrone ring, but it was founded on unsatisfactorily characterised starting material and degradation products and has rightly never been accepted.

In 1949 Clarke, Kidder, and Robertson (*J. Pharm. Pharmacol.*, **1**, 377) purified oenanthotoxin chromatographically and isolated it as colourless crystals, m. p. 80—81°, which decomposed in air to a brown resin. They gave its formula as $C_{18}H_{22}O_4$. Following conversations with Mr. W. D. Robertson, who participated in the above work, we undertook a study of the chemistry of oenanthotoxin. We realised at an early stage that it was a highly unsaturated compound, and the assumption of related properties in cicutoxin then suggested a convenient way for isolating the latter pure. Our studies were consequently widened to embrace both toxins which, as expected, are indeed closely related. Our experiments with them are conveniently described together.

Isolation.—The amounts of oenanthotoxin and ether-soluble contaminants in *Oenanthe crocata* vary seasonally and the method of isolation is best varied accordingly. Fresh roots collected in November or December were used in our first experiments, and these provide

the best starting material. The ethereal extract was purified by shaking it with sodium hydroxide solution, and the product, when treated with carbon tetrachloride, gave crude crystalline material which was readily purified by recrystallisation. The compound so obtained had m. p. 87° and the formula $C_{17}H_{22}O_2$. These values diverge somewhat from those of Clarke and his collaborators, but we confirm their results on the toxicity and instability of the compound and consider their substance was essentially the same as, although a little less pure than, ours. Their incorrect formula was probably largely due to aerial oxidation of their specimen before analysis.

This simple method of isolation failed when applied to the ether-soluble material obtained from fresh roots gathered in April, so the crude oil was analysed chromatographically as described in the Experimental section. A small amount of oenanthotoxin was then obtained, and two other closely related compounds were also isolated pure; these are also present in the autumn plant, but escaped detection in the simple procedure first employed. The first was an alcohol, oenanthetol, $C_{17}H_{22}O$; the second, present only in very small quantity, a ketone, oenanthetone, $C_{17}H_{20}O$. Like oenanthotoxin, both were unstable towards air, and all three compounds had the same very characteristic ultraviolet absorption spectrum (Fig. 1 and Table 3), except for minor differences in intensity.

The nature of this spectrum, together with the realisation that the properties of cicutoxin preparations found by the early workers were consistent with the presence of conjugated unsaturated centres, suggested that cicutoxin too would show long wavelength absorption bands, the intensity of which might be used to control a purification procedure. This view was confirmed when extracts of *Cicuta virosa* were found to show intense and characteristic absorption between 300 and 350 mμ.

The substances responsible for this light absorption were sought by using Brockmann and Volpers' excellent method for the chromatography of colourless substances on aluminium oxide dyed with morin (*Chem. Ber.*, 1947, **80**, 77). Columns of this adsorbent fluoresce when illuminated with ultra-violet light of *ca.* 360 mμ, and those zones which contain material having marked light absorption in the near ultra-violet region then show up as dark bands on a luminous background. In this way, two distinct ultra-violet light absorbing substances were separated from the crude oily extract of *Cicuta* and were purified extensively. The less strongly adsorbed crystallised readily but its complete purification was tedious and wasteful. It furnished a colourless or very faintly yellow alcohol, cicutol, $C_{17}H_{22}O$. The more strongly adsorbed substance was obtained crystalline only with great difficulty; seed crystals were essential for success on subsequent occasions, but if these were available its purification was easier than that of cicutol. The pure material, $C_{17}H_{22}O_2$, isomeric with oenanthotoxin, had essentially the same ultra-violet absorption spectrum as cicutol (Fig. 1; see also Table 3). The name cicutoxin is retained for it, since it had the characteristic convulsant action on mice; cicutol, like oenanthetol, was relatively non-toxic.

All these five compounds formed colourless low-melting crystals which were transformed in air and light into brown insoluble resins. They could be stored unchanged for some weeks below 0° under oxygen-free nitrogen in the dark, and survived recrystallisation from warm solvents and brief heat-treatment during chemical manipulations provided air and light were excluded. Some of their properties are summarised in Table 1.

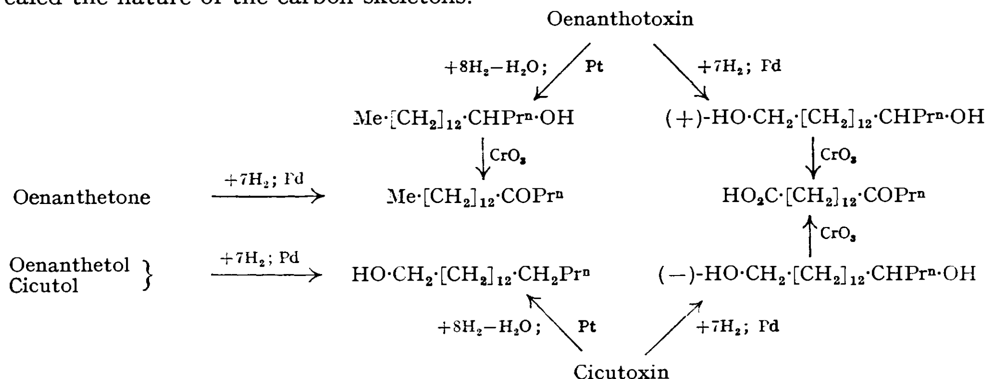
TABLE 1.

	Formula	Toxicity *	M. p.	$[\alpha]_D$
Oenanthotoxin	$C_{17}H_{22}O_2$	+	87°	+30·5° (in MeOH)
Oenanthetol	$C_{17}H_{22}O$	—	71	0 ,,
Oenanthetone	$C_{17}H_{20}O$		46	
Cicutoxin	$C_{17}H_{22}O_2$	+	54	−14·5 (in EtOH)
Cicutol	$C_{17}H_{22}O$	—	66	0 ,,

* 0·2 Mg. suspended in lecithin-saline administered intraperitoneally to a mouse of *ca.* 20 g.

Carbon Skeletons and Oxygen Functions.—Oenanthotoxin and cicutoxin are diols, oenanthetol and cicutol alcohols, and oenanthetone is an unconjugated ketone. The oxygen functions were disclosed by the usual reagents but the products were very difficult

to crystallise, and only one derivative, cicutyl acetate, could be obtained crystalline and pure. The natures of the oxygen functions were first indicated by infra-red spectroscopic evidence. All compounds except oenanthetone showed strong bands near 3300 cm.$^{-1}$; the two toxins showed no bands indicative of oxygen functions other than hydroxyl groups, and their very strong adsorption on aluminium oxide suggested that both contained two such groups. Oenanthetone showed a very strong absorption band at 1704 cm.$^{-1}$. This evidence was confirmed by the hydrogenation experiments summarised below, which also revealed the nature of the carbon skeletons.



With palladium in alcohol oenanthotoxin absorbed 7 mols. of hydrogen, giving a saturated aliphatic diol, $C_{17}H_{34}(OH)_2$, whch formed a di-1-naphthylurethane. With platinum in alcohol about 7·5 mols. of hydrogen were absorbed, giving a mixture of about equal amounts of the same saturated diol and a saturated alcohol, $C_{17}H_{35}\cdot OH$. Chromic acid oxidised the alcohol to a ketone, $C_{17}H_{34}O$, which was degraded by the Schmidt method with hydrazoic and sulphuric acids in chloroform. After the resulting amide mixture had been hydrolysed, tridecane-1-carboxylic acid, *n*-propylamine, and *n*-tridecylamine were isolated. The saturated alcohol must thus be a heptadecan-4-ol and the ketone must be heptadecan-4-one, and this was confirmed by synthesising the latter from myristoyl chloride and di-*n*-propylcadmium.

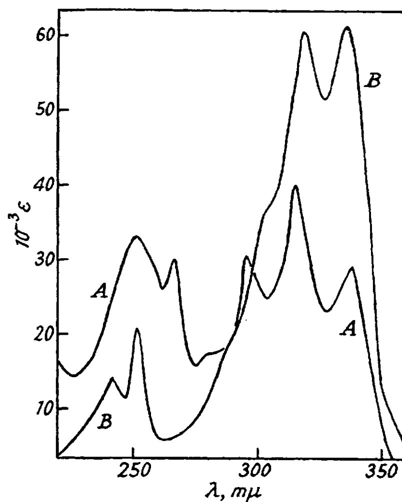


FIG. 1. *Light absorption of oenanthotoxin (A) and cicutol (B) in alcohol.*

Similar methods identified the saturated diol as a heptadecane-1 : 14-diol. On oxidation it gave a keto-acid, $C_{17}H_{32}O_3$, which must be 13-ketohexadecane-1-carboxylic acid, since Schmidt degradation with 1 mol. of hydrazoic acid converted it into an amide mixture which yielded on hydrolysis dodecane-1 : 12-dicarboxylic acid, *n*-propylamine, 12-aminododecane-1-carboxylic acid, and *n*-butyric acid. Since both the alcohol and the diol formed in the hydrogenation with platinum have a hydroxyl group at the same position it is clear that the alcohol was formed by a hydrogenolysis of a primary hydroxyl group at $C_{(1)}$ in oenanthotoxin before saturation. This position must therefore be adjacent to part of the unsaturated system of the toxin.

In the presence of palladium oenanthetol absorbed 7 mols. of hydrogen and gave heptadecan-1-ol, identified by oxidation to hexadecan-1-carboxylic acid. Oenanthetone similarly absorbed 7 mols. of hydrogen and gave the heptadecan-4-one already mentioned. Since all three compounds from *Oenanthe* had the same ultra-violet absorption it is thus probable that oenanthetol is 14-deoxyoenanthotoxin and that oenanthetone is the 14-ketone from 1-deoxyoenanthotoxin.

In methanol with palladium or platinum catalysts cicutoxin absorbed 7 mols. of hydrogen and gave a saturated diol $C_{17}H_{34}(OH)_2$, the enantiamorph of that obtained from oenanthotoxin. Both gave the same 13-ketohexadecane-1-carboxylic acid on oxidation; each had the same m. p. (71°) *, which was depressed on admixture with the other, and neither showed optical activity large enough for detection. The heptadecane-1 : 14-diol from the dextrorotatory oenanthotoxin is here denoted by the prefix *d*-, and that from the lævorotatory cicutoxin by the prefix *l*-, solely for convenience in reference. In acetic acid containing a trace of hydrochloric acid and large amounts of platinum, cicutoxin absorbed nearly 8 mols. of hydrogen; only a small amount of *l*-heptadecane-1 : 14-diol was then formed, the major product being heptadecan-1-ol (m. p. 54°) *. It follows that in cicutoxin $C_{(14)}$, which carries the hydroxyl group eliminated in the above hydrogenolysis, must be immediately adjacent to part of the unsaturated systen.

With a palladium catalyst cicutol took up 7 mols. of hydrogen and formed heptadecan-1-ol; it is therefore probable that cicutol is 14-deoxycicutoxin.

The Chromophores.—The evidence already presented shows that the two unsaturated systems under examination are not of a simple polyene type. If the two toxins contained 7 ethylene links, 6 of them must be conjugated between $C_{(2)}$ and $C_{(13)}$ and both compounds would then have the same ultra-violet absorption as dimethyltetradecahexaene, Me·[CH:CH]$_6$·Me, which absorbs at longer wave-lengths than either (Kuhn, *Angew. Chem.*, 1939, **50**, 703). Infra-red spectroscopic evidence showed that we were dealing with mixed chromophores composed of disubstituted acetylene links and *trans*-ethylene links of the type RCH:CHR'. All five compounds showed bands at about 2200 (RC≡CR' stretching), 2120 (the identity of this band is considered below), 1640 and 1600 (conjugated C=C stretching), and 990 cm.$^{-1}$. The last band is due to the out-of-plane C–H vibrations of the *trans*-RCH:CHR' groups; the normal value for this mode is about 965 cm.$^{-1}$, and the shift to higher frequencies here observed is undoubtedly due to the effect of considerable conjugation. Absorption bands characteristic of allene systems or of *cis*-RCH=CHR' groups were absent. The compounds contained no terminal ethynyl groups since they failed to give silver derivatives.

Both chromophores contain two acetylenic links. This was shown, with oenanthotoxin and cicutol as representatives of each, by partial hydrogenation in the presence of Lindlar's catalyst (*Helv. Chim. Acta*, 1952, **35**, 446), which is selective even for acetylene links which form part of a conjugated system. Figs. 2 and 3 show the changes, similar for both compounds, in the ultra-violet absorption of the two compounds after uptake of 1, 2, and 3 mols. of hydrogen. After 2 mols. of hydrogen had been absorbed the short-wave-length bands had disappeared; the long-wave-length band system had been replaced by a second system, which was typically polyene in type, and both more intense and of a longer wave-length than the original system. The transition to this kind of absorption was incomplete when only 1 mol. of hydrogen had been absorbed. When 3 mols. had been absorbed the intensity of the polyene band system had decreased considerably; the onset of this decrease was apparent when only 2·3 mols. of hydrogen had been absorbed. These changes indicate an initial semi-hydrogenation of two acetylenic links to form two (probably *cis*-)ethylenic links, followed later by a slow general reduction of the whole unsaturated system.

Attempts to isolate the two products of semi-hydrogenation failed, owing to polymerisation. Their light-absorption maxima (Table 2) were very similar, though not identical owing to the different positions of the *cis*-links. Although comparison with the spectra of other unbranched polyenes is complicated by the lack of uniformity in the solvents for which measurements are available, the deviations so caused are unlikely to be large, and it is clear from Table 2 that the products from oenanthotoxin and cicutol both contain a conjugated pentaene system.

* In 1945 M. Sutter and E. Schlittler had extracted *Cicuta virosa* and purified the toxic material extensively; they noticed that the active oily fractions contained highly unsaturated compounds. By hydrogenation of such active extracts they had obtained two crystalline compounds, m. p. 54° and 70°, respectively, but no conclusions could be drawn whether these were derivatives of the physiologically active compounds or not (personal communication from Professor Schlittler).

It follows that both the chromophores under examination contain two acetylene and three ethylene links in conjugation. In both of them too the acetylene links were shown to be adjacent; that is, to form a conjugated diacetylene group. Reaction of oenanthotoxin with methyl iodide and silver oxide gave the dimethyl ether, which did not crystallise, but was readily separated chromatographically from unchanged and partially methylated material, and then showed ultra-violet absorption almost identical with that of the parent compound. When heated with an excess of maleic anhydride the dimethyl ether reacted completely to form an adduct, as shown by the total disappearance of the intense long wave-length absorption bands characteristic of the toxin and the dimethyl ether. Removal of the excess of maleic anhydride was readily effected by partition methods, giving the adduct pure and crystalline. Its ultra-violet absorption maxima were almost identical with those of the dec-2-ene-4 : 6-diyn-1-ol (I) obtained synthetically by Bruun, Haug, and Sörensen (*Acta Chem. Scand.*, 1950, **4**, 850) (see Table 3). The adduct must therefore contain a conjugated ene-diyne system, and have been formed by normal addition of the anhydride to a conjugated diene system situated terminally in the original chromophore.

TABLE 2. *Light absorption of unbranched conjugated polyenes* (*wave-lengths in* mμ).

	$\lambda_{max.}$	$10^{-3}\varepsilon$	$\lambda_{max.}$	$10^{-3}\varepsilon$	$\lambda_{max.}$	$10^{-3}\varepsilon$	$\lambda_{max.}$	$10^{-3}\varepsilon$
$Me\cdot[CH{=}CH]_4\cdot Me$ [a]	272	18	282	36	296	52	320	52
$Me\cdot[CH{=}CH]_6\cdot Me$ [b]	328	25	340	46	360	69	375	50
$Me\cdot[CH{=}CH]_5\cdot Me$ [c]	302	—	312	—	330	—	349	—
Pentaene from oenanthotoxin [d]	300	27	315	39	326	47	343	39
Pentaene from cicutol [d]	302	23	315	44	330	63	347	56

[a] In hexane (Kuhn, *loc. cit.*). [b] In chloroform (*idem, ibid.*). [c] Approx., calc. by interpolation. [d] Measured on the alcoholic solutions obtained by semi-hydrogenation.

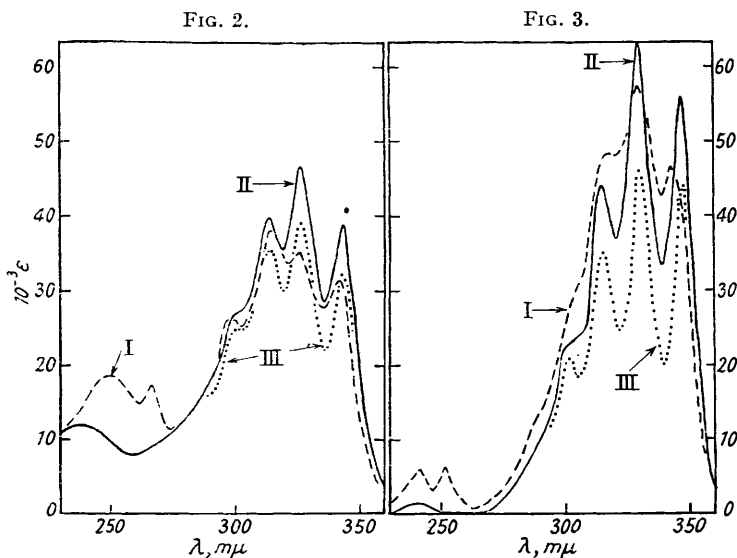
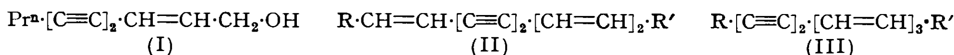


FIG. 2. *Effect of partial hydrogenation on the light absorption of oenanthotoxin in alcohol. After absorption of* (I) 1 *mol.,* (II) 2 *mols., and* (III) 3 *mols. of* H_2.

FIG. 3. *Effect of partial hydrogenation on the light absorption of cicutol in alcohol. After absorption of* (I) 1 *mol.,* (II) 2 *mols., and* (III) 3 *mols. of* H_2.

Cicutoxin gave exactly similar results. The maleic anhydride adduct of its dimethyl ether did not crystallise, but was obtained essentially pure by partition methods; its freedom from unchanged cicutoxin dimethyl ether was shown by the absence of light absorption at 335·5 mμ where the dimethyl ether absorbs very intensely. The ultra-violet absorption maxima of the adduct (Table 3) showed that it contained a conjugated ene-

diyne system and that it must have been formed by normal addition to a conjugated diene system situated terminally in the original chromophore. It follows that one of the two chromophores is that of an ene-diyne-diene (II) and the other that of a triene-diyne (III).



Heilbron, Jones, and their collaborators have compared the ultra-violet absorption spectra of polyenynes of the types $R\cdot[CH{:}CH]_n\cdot C{\vdots}CH$ and $R\cdot[CH{:}CH]_n\cdot C{\vdots}C\cdot[CH{:}CH]_n\cdot R$ with those of polyenes with the same number of unsaturated centres (*J.*, 1943, 261, 268; 1944, 134, 136). Their results show that the acetylene link causes a diminution in intensity

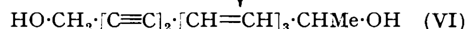
TABLE 3. *Light absorption in alcohol* (*wave-lengths* in mμ).

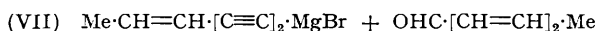
	$\lambda_{max.}$	$10^{-3}\,\varepsilon$		$\lambda_{max.}$	$10^{-3}\,\varepsilon$
Oenanthotoxin	213	17·5	Cicutoxin	242	13
	252	33		252	22
	267	29		287 *	17
	281 *	17·5		303 *	38·5
	296	30·5		318·5	63·5
	315·5	40		335·5	65
	337·5	29			
Maleic anhydride adduct of oenanthotoxin Me_2 ether	241	8·7	Maleic anhydride adduct of cicutoxin Me_2 ether	241	6·6
	254	16·0		254	13·7
	268	23·0		269	18·9
	284	17·9		284	15·4
Dec-2-ene-4 : 6-diyn-1-ol (I)	239·5	7·3			
	252	14·6			
	266·5	21·8			
	282·5	19·4			

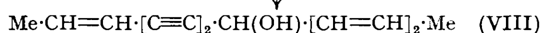
* Inflexion.

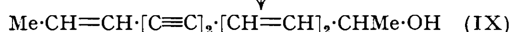
and a shift towards shorter wave-lengths of the main absorption band; the reduction in intensity is greater when the acetylenic link is centrally disposed than when it is terminal in the chromophore. If, as seems probable, the effect of a conjugated diyne system is similar, then the light absorption data indicate that the compounds from *Oenanthe* have the ene-diyne-diene chromophore (II) and those from *Cicuta* the triene-diyne chromophore (III), since the latter show long wave-length absorption of very high intensity. These conclusions were confirmed by syntheses of compounds containing the chromophores (II) and (III).

The methods chosen were suggested by the conjugated poly-yne syntheses described by Bohlmann (*Chem. Ber.*, 1951, **84**, 545, 785) and by Jones (Tilden Lecture, *J.*, 1950, 754; for recent fuller accounts of the extensive work of Jones and his co-workers, see *Nature*, 1951, **168**, 900; *J.*, 1952, 1993—2014). In the first of our syntheses, penta-2 : 4-diyn-1-ol, obtained by interaction of formaldehyde and monosodiodiacetylene (Bohlmann, *loc. cit.*) was converted into its dimagnesium bromide derivative (IV) and condensed with octatrienal









to give the crude diol (V), which when shaken in ether with dilute acid rearranged and furnished the crystalline trideca-6 : 8 : 10-triene-2 : 4-diyne-1 : 12-diol (VI). In the second

synthesis, hept-2-ene-4 : 6-diyne, in the form of its magnesium bromide (VII), was condensed with sorbaldehyde to give the crude alcohol (VIII) from which, after rearrangement in aqueous acidic methanol, crystalline trideca-3 : 5 : 11-triene-7 : 9-diyn-2-ol (IX) was obtained, together with some of its methyl ether.

The ultra-violet absorption spectrum of the triene-diyne (VI) was almost identical with that of cicutoxin (see Fig. 5), confirming that they have the same chromophore; similarly the ene-diyne-diene (IX) and oenanthotoxin had almost identical extinction curves (Fig. 4). The infra-red absorption spectra of the synthetic compounds showed the expected features; in particular they showed a very strong band near 990 cm.$^{-1}$ but none near 690 cm.$^{-1}$, showing that, like the natural compounds, their ethylenic links were all-*trans*.

The structures of the two unsaturated systems being known, two minor points require mention. The presence of a conjugated diacetylene system probably explains the presence of a weak band near 2120 cm.$^{-1}$ in the infra-red spectra of all five natural compounds.

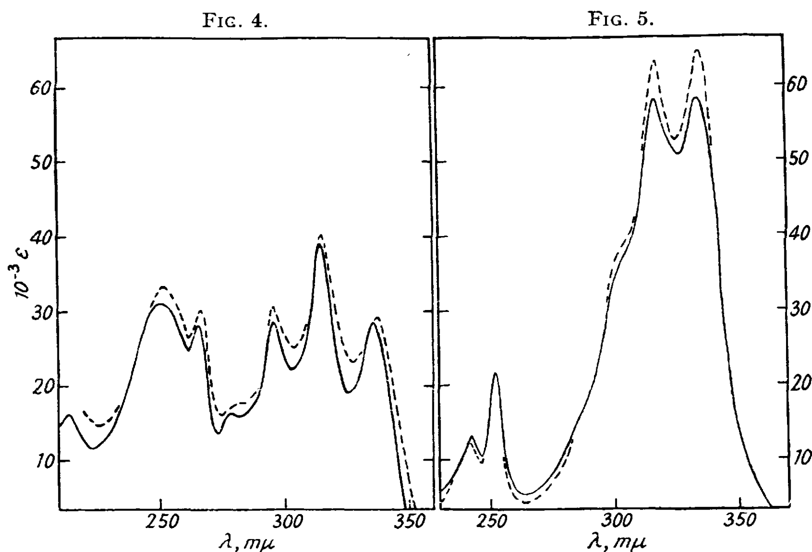


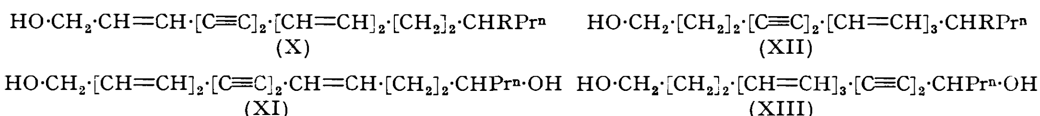
FIG. 4. *Light absorption of*
—— *Trideca-3 : 5 : 11-triene-7 : 9-diyn-2-ol.* – – – – *Oenanthotoxin.* (*Both in alcohol.*)
FIG. 5. *Light absorption of*
—— *Trideca-6 : 8 : 10-triene-2 : 4-diyn-1 : 12-ol.* – – – – *Cicutoxin.* (*Both in alcohol.*)

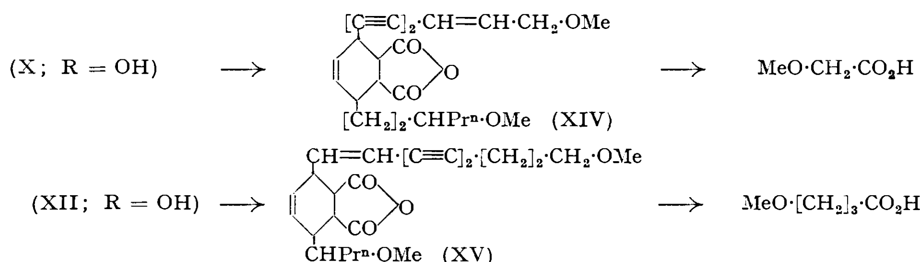
Dimethyldiacetylene shows bands at 2264 and 2157 cm.$^{-1}$ which are due respectively to the symmetrical and unsymmetrical stretching modes of the acetylene links (see Sheppard and Simpson, *Quart. Reviews*, 1952, **6**, 1), so the band near 2120 cm.$^{-1}$ may well represent the unsymmetrical stretching mode of the diacetylene system. The second point concerns the spacing of the long-wave-length ultra-violet absorption bands in the ene-diyne-dienes (*ca.* 2100 cm.$^{-1}$) and in the triene-diynes (*ca.* 1600 cm.$^{-1}$). In chromophores containing conjugated di- and poly-acetylene links the relevant spacings frequently correspond to the stretching frequency of the acetylene links, a fact which may facilitate the identification of such chromophores in natural products (Bruun, Haug, and Sörensen, *loc. cit.*; Jones *et al.*, *Nature*, 1951, **168**, 900; Anchel, *J. Amer. Chem. Soc.*, 1952, **74**, 1588). The spacing observed in oenanthotoxin fits well into this generalisation, but that observed in cicutoxin corresponds to the stretching frequency of the ethylene, not the acetylene links. It is to be expected that as the proportion of ethylene to acetylene links in a mixed chromophore increases such behaviour will become more prominent, especially where the acetylene links are terminal in the chromophore; in such cases, too, the intensity of the long-wave-length absorption corresponds more closely to that characteristic of purely ethenoid systems.

Position and Orientation of the Chromophores.—In oenanthotoxin and oenanthetol the unsaturated system extends from $C_{(2)}$ to $C_{(11)}$ inclusive; for the toxin this was shown by the

hydrogenolysis of the hydroxyl group at $C_{(1)}$ in the presence of platinum, and for oenanthetol by the isolation of *n*-heptoic acid after complete oxidation with potassium permanganate. In cicutoxin and cicutol the unsaturation extends from $C_{(4)}$ to $C_{(13)}$ inclusive; for the toxin this was shown by the platinum hydrogenolysis of the hydroxyl group at $C_{(14)}$, and for cicutol by the isolation of *n*-valeric and succinic acids after complete oxidation with permanganate. Both unsaturated systems being unsymmetrical, they could be oriented in their respective molecules in two ways, so that oenanthotoxin might be (X; R = OH) or (XI) and cicutoxin might be (XII; R = OH) or (XIII).

Two methods were used in attempts to decide between these alternatives. If the hydroxyl group at $C_{(1)}$ in oenanthotoxin could be oxidised to the aldehyde or acid stage, a new chromophore would be created which could be identified spectroscopically with the aid of synthetic models. Such oxidations, however, could be effected neither with chromic acid nor with manganese dioxide in non-polar media; both reagents caused more extensive oxidation, and no trace of the desired oxidation products could be found in spite of the sensitive methods available for their detection. An alternative approach, based on the maleic anhydride adducts mentioned earlier, was more successful.





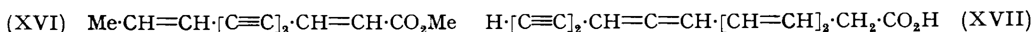
Permanganate oxidation of the crystalline adduct from oenanthotoxin dimethyl ether gave a monobasic acid fraction which, when chromatographed on paper (Long, Quayle, and Stedman, *J.*, 1951, 2197), ran as a single spot, identical in position with that given by methoxyacetic acid. This acid was also isolated from the fraction as its benzylamine salt. Its formation as the sole monobasic acid in the oxidation requires the adduct to have the structure (XIV), and oenanthotoxin to correspond to (X; R = OH); a substance of structure (XI) would have formed an adduct which would have given on oxidation γ-methoxy-*n*-heptoic acid, not methoxyacetic acid.

Similar oxidation of the syrupy but carefully purified adduct from cicutoxin dimethyl ether gave as the sole monobasic acid γ-methoxy-*n*-butyric acid, as shown by paper chromatography and also by the isolation of its crystalline piperazine salt; α-methoxy-*n*-valeric acid, which would have been formed instead if cicutoxin had the structure (XIII) was shown to be absent. It follows that the adduct has the structure (XV) and cicutoxin the structure (XII; R = OH).

The orientations of the chromophores in oenanthetol and cicutol have not been directly determined, but it appears almost certain that they are the same as those in the respective diols, leading to the structure (X; R = H) for oenanthetol and to (XII; R = H) for cicutol. It is of interest that in all these compounds the diacetylene system has the same position in the molecule.

The compounds from *Oenanthe* and *Cicuta* belong to the rapidly growing class of naturally occurring poly-ynes, recently investigated representatives of which include matricaria ester (XVI) and dehydromatricaria ester (Holman and Sörensen, *Acta Chem. Scand.*, 1950, **4**, 416; Stavholt and Sörensen, *ibid.*, pp. 1567, 1575), mycomycin (XVII) (Celmer and Solomons, *J. Amer. Chem. Soc.*, 1952, **74**, 1870), and the antibiotics of as yet

undetermined structures which occur in representatives of the Basidiomycetes (Anchel, *loc. cit.*). The present compounds are the first alcohols of the group, and other features of interest include the very uncommon *n*-heptadecane skeletons, the toxicity of the diols, and its virtual disappearance in the mono-ols.


(XVI) Me·CH=CH·[C≡C]$_2$·CH=CH·CO$_2$Me H·[C≡C]$_2$·CH=C=CH·[CH=CH]$_2$·CH$_2$·CO$_2$H (XVII)

A summary of some of the above work has already appeared (*Chem. and Ind.*, 1952, **31**, 757). Experiments on the synthesis of the natural compounds and their analogues are in progress.

EXPERIMENTAL

Whenever possible, operations with unsaturated compounds were conducted in diffuse light under oxygen-free nitrogen; evaporations were carried out below 36°, under reduced pressure when necessary. Solvents were carefully purified before use; light petroleum refers to the fraction of b. p. 40—60°, except where otherwise specified. Morin–aluminium oxide refers to material of activity grade II, prepared by Brockmann and Volpers' method (*loc. cit.*).

Isolation of Oenanthotoxin.—Fresh roots of *Oenanthe crocata* (8 kg.), collected in December, were minced and extracted (Soxhlet) with ether (7 l.) for 12 hours. After it had been separated from accompanying water and sludge, the ethereal extract was concentrated to 150 c.c. and shaken with 20% sodium hydroxide solution, which precipitated much colloidal matter. The precipitate was removed and washed with ether, and the combined ethereal solutions shaken with more sodium hydroxide solution, then washed with saturated sodium sulphate solution, dried, and evaporated. The residual oil (10 g.) was dissolved in a small amount of benzene, and warm carbon tetrachloride (35 c.c.) was added, followed by enough light petroleum (b. p. 60—80°) to render the solution just turbid. After 2 days at 0° the crude yellow crystals (3 g.), m. p. 77—80°, were collected and recrystallised successively from benzene, chloroform–light petroleum, methanol, and finally ether, giving *oenanthotoxin* as large prisms, m. p. 87°, $[\alpha]_D^{15}$ +30·5° (*c*, 2·0 in MeOH) [Found: C, 78·9; H, 8·8%; *M* (ebullioscopic in $CHCl_3$), 245, 275. $C_{17}H_{22}O_2$ requires C, 79·1; H, 8·6%; *M*, 258]. Light absorption: see Fig. 1 and Table 3. The infra-red spectrum showed, *inter al.*, bands at 3390 and 3268 (strong), 2193 and 2123 (v. weak), 1639 (medium), 1590 (weak), and 986 cm.$^{-1}$ (v. strong).

Isolation of Oenanthetol and Oenanthetone.—Fresh roots of *Oenanthe* (7 kg.), collected in March—April, were treated as before, but no crystalline material could be obtained. The combined ether-soluble material (100 g.) from 36 kg. of roots was dissolved in benzene (80 c.c.), the solution shaken with light petroleum (400 c.c.), and the oily precipitate removed and shaken with more light petroleum. The insoluble fraction was extracted with carbon tetrachloride, giving a soluble fraction 1*c* (12 g.) and an insoluble fraction 1*d* (57 g.). The combined light petroleum extracts were evaporated, and the residue dissolved in pentane (250 c.c.) and cooled to −15°, whereupon a gum (fraction 1*b*; 8·7 g.) separated and was removed. Evaporation of the pentane solution gave fraction 1*a* (21·8 g.).

Fraction 1*a* was chromatographed in light petroleum (100 c.c.) on morin–aluminium oxide (1·3 kg.; deactivated with 65 c.c. of water) and the chromatogram developed with the same solvent (2·5 l.). Ultra-violet light of 360 mμ showed a broad upper band which travelled very slowly and a lower narrow band which travelled rapidly. Development was continued with benzene–light petroleum (1 : 1), and the filtrate corresponding to the lower band was collected and evaporated, giving fraction 2*a* (820 mg.). The column was cut and the part corresponding to the upper band was eluted with methanol, evaporation of which gave fraction 2*b* (7·4 g.). Similar chromatography of fraction 1*c* gave two fractions absorbing ultra-violet light—a more (2*d*; 13 g.) and a less strongly adsorbed fraction (2*c*; 4 g.).

Fractions 1*b*, 2*b*, and 2*c* were combined and chromatographed on morin–aluminium oxide (1·2 kg.; deactivated with 72 c.c. of water), and the chromatogram developed with light petroleum–benzene (4 : 1 ⟶ 1 : 1). After 3·5 l. of solvent had been collected a band absorbing ultra-violet light commenced to emerge into the filtrate, which was collected separately (1·2 l.) till desorption of the band was complete. Evaporation gave a pale yellow oil (5·75 g.). When its solution in light petroleum had been kept at 0° for a few days crude crystalline material had separated; recrystallisation from light petroleum gave *oenanthetol* (1·1 g.), m. p. 71° (Found: C, 84·4; H, 9·5. $C_{17}H_{22}O$ requires C, 84·3; H, 9·2%). Light absorption in EtOH: max. at 213, 252, 267, 281, 297, 316, and 338 mμ; $10^{-3}\varepsilon$, 17·5, 30·8, 28·0, 17·0, 28·4, 37·6, and 27·1. The

infra-red spectrum showed *inter al.* bands at 3257 (medium strong), 2193 (v. weak), 2114 (v. weak), 1631 (medium strong), 1587 (weak), and 985 cm.$^{-1}$ (v. strong).

Fraction 2*a* was chromatographed on morin–aluminium oxide (90 g.; deactivated with 6 c.c. of water), and the chromatogram developed with light petroleum. The filtrate corresponding to the broad, fast-moving band was collected separately and evaporated, giving an oil which crystallised when cooled. Recrystallisation from pentane gave *oenanthetone* (50 mg.) as plates, m. p. 46° (Found: C, 85·1; H, 8·3. $C_{17}H_{20}O$ requires C, 85·0; H, 8·4%). Light absorption in EtOH: max. at 251, 267, 280, 296, 315, and 337 mμ; 10^{-3} ε, 30·0, 25·9, 15·0, 27·0, 37·0, and 27·0. The infra-red spectrum showed *inter al.* bands at 2188 (v. weak), 2120 (v. weak), 1704 (v. strong), 1629 (medium), 1582 (v. weak), and 986 cm.$^{-1}$ (v. strong).

By chromatography of fractions 1*d* and 2*d*, oils absorbing ultra-violet light were obtained which on cooling deposited small quantities of crystals, recrystallisation of which gave oenanthotoxin.

Isolation of Cicutoxin and Cicutol.—Fresh rhizomes of *Cicuta virosa* (17 kg.; collected in February) were minced and extracted (Soxhlet) in portions (*ca.* 6 kg.) with ether (8 l.) for 16 hours. After separation from water and sludge the combined extracts were dried and evaporated, and the residue was dissolved in benzene (500 c.c.) and filtered through Hyflo Supercel silica. Evaporation gave a dark brown oil (120 g.) with a terpene-like odour. A portion (15 g.) was chromatographed in benzene (50 c.c.) on morin–aluminium oxide (600 g.), and the column washed with methanol–benzene (1 : 100); when *ca.* 2·5 l. of filtrate had been collected, examination in ultra-violet light revealed three dark zones. The top one was stationary close to the top of the column, the central moved slowly, and the lowest moved rapidly. The filtrate (1 l.) corresponding to the latter was collected separately and evaporated, giving a brown oil (fraction 1; 5·0 g.) which partly solidified at room temperature. After a further 500 c.c. of filtrate had been collected, the next dark band commenced to emerge; the filtrate (2·5 l.) was then collected separately and evaporated, giving a brown gum (fraction 2; 2·5 g.). Elution of the upper zone with methanol and evaporation gave a very dark and dirty gum without the selective light absorption characteristic of the other two zones; it was discarded.

Fraction 1 was adsorbed from benzene (50 c.c.) on morin–aluminium oxide (200 g.), and the chromatogram developed with methanol–benzene (1 : 100). In ultra-violet light a single broad dark zone was seen; in visible light this zone was made up of three parts; leading and trailing edges appeared as narrow brown bands, enclosing a colourless zone. The filtrate corresponding to the last was collected separately; evaporation gave a pale yellow oil (3·4 g.) which solidified completely at room temperature. Crystallisation from light petroleum gave crude material (2·5 g.), m. p. 53—57°. Repeated recrystallisation from benzene–light petroleum and from carbon tetrachloride–light petroleum gave *cicutol* as large, almost colourless plates, m. p. 66°, $[\alpha]_D^{15}$ 0° (*c*, 1·5 in EtOH) (Found: C, 84·4; H, 9·5. $C_{17}H_{22}O$ requires C, 84·3; H, 9·2%). Light absorption in EtOH: max. at 242, 252, 318·5, and 335 mμ: inflections at 287 and 302 mμ; 10^{-3} ε, 14·1, 20·8, 60·6, 61·2, 17·5, and 35·6. The infra-red spectrum showed *inter al.* bands at 3333 (strong), 2212 (medium), 2128 (weak), 1634 (weak), 1603 (medium), and 996 cm.$^{-1}$ (v. strong). With a palladium–charcoal catalyst in alcohol cicutol absorbed 7·2 mols. of hydrogen. Acetylation with pyridine–acetic anhydride at room temperature in the usual manner, and crystallisation of the product from a small volume of light petroleum, gave *cicutyl acetate* as needles, m. p. 38—39·5° (Found: C, 80·0; H, 8·4. $C_{19}H_{24}O_2$ requires C, 80·3; H, 8·4%).

Fraction 2 was adsorbed from benzene (50 c.c.) on morin–aluminium oxide (100 g.), and the chromatogram developed with methanol–benzene (1 : 100). Ultra-violet light revealed a single broad dark zone, the leading edge of which appeared in visible light as a brown band. The filtrate corresponding to the latter was discarded, and that corresponding to the rest of the zone collected separately (*ca.* 2·5 l.) and evaporated. The yellow gum (1·5 g.) so obtained was spectroscopically *ca.* 70% pure; such material could be crystallised directly if seed crystals were available. One such sample crystallised spontaneously at 0° in 5 days, and the crystals were drained from oil and carefully preserved. The following directions are given for the crystallisation, which is not easy. The gum (1·5 g.), dissolved in just enough benzene (*ca.* 20 c.c.) to give a solution clear at 10° but turbid at 7°, was treated at 10° with enough light petroleum to cause marked turbidity, which was removed by addition of ether. The solution was then seeded and kept at 8—9° for 1 hour, whereupon crystallisation usually commenced; if it did not, the operation was repeated with a *larger* volume of solvent. The solution was then cooled progressively to 0° during 6 hours, then kept at 0° for 12 hours, and the dense crystalline deposit (0·9 g.), m. p. 45—49°, collected. Recrystallisation in portions (100 mg.) from ether–light

petroleum gave *cicutoxin* (0·7 g.) as prisms, m. p. 54°, $[\alpha]_D^{15}$ −14·5° (*c*, 1·7 in EtOH) (Found : C, 78·8; H, 8·7. $C_{17}H_{22}O_2$ requires C, 79·1; H, 8·6%). Light absorption : see Fig. 1 and Table 3. The infra-red spectrum showed *inter al.* bands at 3226 (strong), 2212 (weak), 2128 (v. weak), 1634 (v. weak), 1603 (medium), and 996 cm.$^{-1}$ (v. strong).

Hydrogenation of Oenanthotoxin with Palladium.—The toxin (500 mg.) was hydrogenated in alcohol (30 c.c.) with 2·5% palladium–barium sulphate (270 mg.) at room temperature and pressure (uptake, 6·84 mols.). The product, isolated in the usual way, was chromatographed on aluminium oxide and crystallised from chloroform–light petroleum, giving d-*heptadecane*-1 : 14-*diol* (430 mg.) as needles, m. p. 71°, $[\alpha]_D^{12}$ 0° ± 1° (*c*, 2·0 in MeOH) (Found : C, 75·3; H, 13·3. $C_{17}H_{36}O_2$ requires C, 75·0; H, 13·3%). The *bis*-1-*naphthylurethane* separated from chloroform–light petroleum as needles, m. p. 81—82° (Found : C, 76·7; H, 8·1; N, 4·7. $C_{39}H_{50}O_4N_2$ requires C, 76·7; H, 8·2; N, 4·6%).

Hydrogenation of Oenanthotoxin with Platinum.—The toxin (1 g.) was hydrogenated as above in alcohol (50 c.c.) with Adams' platinum catalyst (60 mg.) (uptake, 7·6 mols.). The product was chromatographed on deactivated aluminium oxide, light petroleum with benzene, and later benzene alone, being used for development. The less strongly adsorbed material, namely, *heptadecan*-4-*ol* (440 mg.), crystallised from methanol as needles, m. p. 53°, $[\alpha]_D^{12}$ 0° ± 1° (*c*, 2·0 in MeOH) (Found : C, 80·1; H, 13·9. $C_{17}H_{36}O$ requires C, 79·7; H, 14·0%). The 1-*naphthylurethane* crystallised from pentane as needles, m. p. 82—83° (Found : C, 79·0; H, 10·3; N, 3·4. $C_{28}H_{43}O_2N$ requires C, 79·0; H, 10·2; N, 3·3%).

Crystallisation of the more strongly adsorbed material from the chromatogram from chloroform–light petroleum gave *d*-heptadecane-1 : 14-diol (535 mg.), m. p. 71°, undepressed on admixture with the material described above.

Oxidation of Heptadecan-4-*ol.*—The alcohol (200 mg.), potassium dichromate (200 mg.), concentrated sulphuric acid (250 mg.), and water (0·9 c.c.) were stirred together at 50° for 20 minutes, cooled, and diluted with water (2 c.c.), and the product isolated with ether in the usual manner. Recrystallisation from methanol gave heptadecan-4-one (140 mg.) as plates, m. p. 41—42°; Oldham and Ubbelohde (*J.*, 1939, 201) give m. p. 41·5°.

Schmidt Degradation of Heptadecan-4-*one.*—To a stirred solution of the ketone (1·8 g.) in chloroform (15 c.c.) containing concentrated sulphuric acid (5 c.c.) a 1·15N-solution of hydrazoic acid in chloroform (6·6 c.c.) was added slowly at 0°. Stirring was continued a further 15 minutes, crushed ice and more chloroform were added, and the chloroform layer was washed successively with water, 5% aqueous potassium hydroxide, and finally water, then dried and evaporated. The crystalline mixture of amides (1·5 g.) remaining had m. p. *ca.* 63°. A portion (0·5 g.) and 48% hydrobromic acid (10 c.c.) were boiled under reflux for 24 hours, then diluted and cooled to 0°. Tridecylamine hydrobromide which separated was characterised as N-*phenyl*-N'-*tridecylthiourea*, m. p. 78° (Found : N, 8·6. $C_{20}H_{34}N_2S$ requires N, 8·4%). The filtrate was extracted with ether and the aqueous phase made alkaline and treated with phenyl *iso*thiocyanate, which gave *N*-phenyl-*N'*-*n*-propylthiourea, m. p. 62°, undepressed on admixture with authentic material prepared from *n*-propylamine. The ethereal extract was extracted with dilute aqueous potassium hydroxide, and the alkaline solution acidified, giving a crystalline precipitate which smelled strongly of *n*-butyric acid. Recrystallisation from acetone gave tridecane-1-carboxylic (myristic) acid, m. p. 54°, undepressed on admixture with authentic material. The anilide had m. p. 84° (Found : N, 4·9. Calc. for $C_{20}H_{33}ON$: N, 4·6%).

Synthesis of Heptadecan-4-*one.*—A solution of *n*-propylmagnesium bromide [from *n*-propyl bromide (4·5 g.), magnesium (0·8 g.) and ether (15 c.c.)] was stirred at 0° for ½ hour with anhydrous cadmium chloride (4 g.), and to the stirred suspension tridecane-1-carboxyl chloride (6·5 g.) in ether (25 c.c.) was added slowly. The mixture was heated under reflux for ½ hour, cooled, and poured on crushed ice and dilute sulphuric acid; the ethereal layer was washed successively with water, dilute aqueous potassium carbonate, and water, dried, and evaporated. Crystallisation of the residue from pentane at −30° and recrystallisation from methanol gave the ketone (40%), m. p. 41—42°, undepressed on admixture with material obtained as described above (Found : C, 80·3; H, 13·7. Calc. for $C_{17}H_{34}O$: C, 80·3; H, 13·5%).

Oxidation of d-*Heptadecane*-1 : 14-*diol.*—A mixture of chromic oxide (150 mg.), water (9 c.c.), and concentrated sulphuric acid (1·2 c.c.) was slowly added to a stirred solution of the diol (200 mg.) in acetone (5 c.c.), and the mixture stirred for ½ hour at room temperature, then diluted with water and extracted with ether. The acidic fraction of the product, isolated in the usual manner, crystallised from light petroleum and was recrystallised from ether–light petroleum, giving 13-*ketohexadecane*-1-*carboxylic acid* (100 mg.) as needles, m. p. 79—80° (Found : C, 71·5; H, 11·6. $C_{17}H_{32}O_3$ requires C, 71·8; H, 11·4%).

Schmidt Degradation of 13-*Ketohexadecane*-1-*carboxylic Acid*.—A 1·15N-solution of hydrazoic acid in chloroform (0·66 c.c.) was added slowly at 0° to a stirred solution of the keto-acid (180 mg.) in chloroform (2 c.c.) containing concentrated sulphuric acid (0·5 c.c.). Stirring was continued for a further 15 minutes and then ice (4 g.) and more chloroform were added. The chloroform layer was washed with water, dried, and evaporated and the residue boiled under reflux with 48% hydrobromic acid (2 c.c.) for 24 hours. The cooled hydrolysate was diluted with water (10 c.c.) and kept at 0°. 12-*Aminododecane*-1-*carboxylic acid hydrobromide* separated as needles (Found, in material dried at 60°/1 mm.: C, 50·0; H, 8·9; N, 4·8. $C_{13}H_{28}O_2NBr$ requires C, 50·3; H, 9·1; N, 4·5%). The filtrate was extracted with ether, evaporation of which left a solid contaminated by *n*-butyric acid (smell). Recrystallisation from ethyl acetate gave dodecane-1 : 12-dicarboxylic acid (50 mg.), m. p. 125° (Found: C, 65·2; H, 10·5. Calc. for $C_{14}H_{26}O_4$: C, 65·1; H, 10·2%). The aqueous phase from the ether-extraction was made alkaline and treated with phenyl *iso*thiocyanate, giving *N*-phenyl-*N'*-*n*-propylthiurea, m. p. 63°.

Hydrogenation of Oenanthetol.—Oenanthetol (90 mg.) in alcohol (20 c.c.) was hydrogenated in the presence of 2·5% palladium–barium sulphate (uptake, 6·8 mols.). The product, isolated in the usual manner, crystallised from light petroleum, giving heptadecan-1-ol (70 mg.) as plates m. p. 54°, undepressed on admixture with material obtained by hydrogenating cicutol (Found: C, 79·2; H, 14·1. Calc. for $C_{17}H_{36}O$: C, 79·7; H, 14·0%). Levene, West, and van der Scheer (*J. Biol. Chem.*, 1915, **20**, 524) give m. p. 54°. Oxidation in acetone with chromic and sulphuric acids gave hexadecane-1-carboxylic (margaric) acid, m. p. 61°. Le Sueur (*J.*, 1904, **85**, 827) gives m. p. 60—61°.

Hydrogenation of Oenanthetone.—The ketone (15 mg.) was hydrogenated in alcohol (15 c.c.) with palladium–barium sulphate (uptake, 6·7 mols.). The product crystallised from methanol, giving heptadecan-4-one, m. p. 42°, undepressed on admixture with authentic material.

Hydrogenation of Cicutoxin with Palladium.—Cicutoxin (190 mg.) was hydrogenated in methanol (20 c.c.) at room temperature and pressure with 5% palladium–charcoal (50 mg.). The product, isolated in the usual manner, was recrystallised from light petroleum, giving l-*heptadecane*-1 : 14-*diol* (123 mg.), m. p. 70—71°, depressed to *ca.* 65° on admixture with the *d*-isomer (Found: C, 75·2; H, 13·6. $C_{17}H_{36}O_2$ requires C, 75·0; H, 13·3%). A chromatographic examination of the mother-liquor furnished more diol (40 mg.), but no monohydroxylic compound was detected.

Oxidation of the *l*-diol as described for the *d*-isomer gave 13-ketohexadecane-1-carboxylic acid, m. p. 80—81°, undepressed on admixture with material obtained from the *d*-isomer.

Hydrogenation of Cicutoxin with Platinum in Acetic Acid.—The toxin (150 mg.) in acetic acid (40 c.c.) containing concentrated hydrochloric acid (0·04 c.c.) and Adams's platinum catalyst (100 mg.) was hydrogenated at room temperature and pressure (uptake, 7·95 mols.). The product, isolated in the usual manner, was extracted with cold light petroleum (3 × 7 c.c.). The insoluble residue crystallised from hot light petroleum, giving *l*-heptadecane-1 : 14-diol (7 mg.) as needles, m. p. 70—71°. Evaporation of the light petroleum extract and crystallisation from light petroleum (3 c.c.) at 0° gave heptadecan-1-ol (110 mg.) as plates m. p. 53—54°, undepressed on admixture with material obtained from the hydrogenation of cicutol.

Hydrogenation of Cicutol.—Cicutol (43 mg.) in alcohol (40 c.c.) was hydrogenated with 5% palladium–charcoal (uptake, 7·2 mols.). The product separated from light petroleum (1 c.c.) at 0° as plates (31 mg.) (heptadecan-1-ol), m. p. 54° (Found: C, 79·6; H, 14·2%). Oxidation with chromic and sulphuric acids in acetone gave hexadecane-1-carboxylic acid, m. p. 61° (Found: C, 75·4; H, 12·5. Calc. for $C_{17}H_{34}O_2$: C, 75·5; H, 12·6%).

Reaction of Oenanthotoxin Dimethyl Ether with Maleic Anhydride.—Oenanthotoxin (620 mg.), methyl iodide (90 c.c.), and silver oxide (6 g.) were heated together under reflux with exclusion of light for 24 hours, and fresh silver oxide (2 × 3 g.) was added after 6 and 12 hours. The mixture was filtered, the residue was washed with benzene, the combined filtrate and washings were evaporated, and the residue was chromatographed in benzene on morin–aluminium oxide. Development with benzene caused a homogeneous zone, dark when viewed in ultra-violet light, to travel rapidly down the column. Evaporation of the corresponding filtrate gave the dimethyl ether as an almost colourless gum (700 mg.) with the following light absorption in EtOH: max. at 252, 267, 296, 316, and 337 mμ; 10^{-3} ε, 31·5, 29·0, 29·0, 38·3, and 27·6. The gum was heated with maleic anhydride (850 mg.) at 100° for 2 hours, and the cooled mixture dissolved in the top layer (100 c.c.) formed by shaking light petroleum, benzene, methanol, and water (1 : 1 : 1 : 1). The solution was washed with the bottom layer (2 × 100 c.c.) from the same system, and the combined washings were shaken again with more of the top layer (20 c.c.). The united top layers were evaporated, crystallisation of the residue from ether–light petroleum

giving the *adduct* (680 mg.) as colourless needles, m. p. 88·5—89° (Found : C, 72·0; H, 7·5. $C_{23}H_{28}O_5$ requires C, 71·9; H, 7·2%). Light absorption : see Table 3.

Reaction of Maleic Anhydride with Cicutoxin Dimethyl Ether.—Cicutoxin (340 mg.) was methylated for 4½ hours and the product purified as described for oenanthotoxin. The dimethyl ether formed a colourless gum (342 mg.) with the following light absorption in EtOH : max. at 242, 251, 318, and 336 mμ : $10^{-3}\,\varepsilon$, 15·6, 21·0, 58·0, and 56·8. It was kept at 100° for 1½ hours with maleic anhydride (468 mg.), and the cooled product partitioned between light petroleum, benzene, methanol, and water (1 : 1 : 1 : 1), as described in the case of oenanthotoxin, in order to remove the excess of maleic anhydride. The adduct (395 mg.) could not be crystallised, but its light absorption at 335·5 mμ showed that it contained $<\frac{1}{2}$% of unchanged cicutoxin dimethyl ether (see also Table 3).

Permanganate Oxidation of Oenanthetol.—Finely powdered potassium permanganate (4·5 g.) was added in portions during 1 hour to a well-stirred solution of oenanthetol (500 mg.) in purified acetone (60 c.c.) at room temperature. Stirring was continued for a further 5 hours at room temperature and then under reflux for 40 minutes, all the permanganate being then reduced. The cooled mixture was filtered and the residue washed, first with ice-water and then with hot water. The combined aqueous filtrates were united with the residue obtained by evaporation of the acetone filtrate, acidified strongly with hydrochloric acid, and extracted with ether (7 × 60 c.c.). After the extract had been washed with water (35 c.c.) it was extracted with saturated aqueous sodium hydrogen carbonate (3 × 25 c.c.). The extract was acidified and the product isolated with ether. It formed a brown gum, which was extracted with cold benzene (2 × 10 c.c.); evaporation of the solvent and distillation of the residue under reduced pressure gave crude *n*-heptoic acid (58 mg.), characterised as the piperazine salt (Pollard, Adelson, and Bain, *J. Amer. Chem. Soc.*, 1934, **56**, 1759), needles (from acetone), m. p. 95°, undepressed on admixture with authentic material of m. p. 95—96° (Found : N, 8·4. Calc. for $C_{18}H_{38}O_4N_2$: N, 8·1%).

Permanganate Oxidation of Cicutol.—Cicutol (660 mg.) in purified acetone (60 c.c.) was oxidised with potassium permanganate (5·8 g.) in the same way as oenanthetol. The monobasic acid fraction, isolated similarly, was identified as *n*-valeric acid (130 mg.) by conversion into the piperazine salt (100 mg.), needles (from acetone), m. p. 109° undepressed on admixture with authentic material (Found : N, 9·8. Calc. for $C_{14}H_{28}O_4N_2$: N, 9·8%). Pollard *et al.* (*loc. cit.*) give m. p. 112·5—113°. The aqueous acidic solution from which the valeric acid had been extracted was evaporated under reduced pressure and the residue dried by distillation with benzene and then extracted with hot ether. Evaporation of the extract left a semi-solid residue which was dissolved in an excess of 0·2N-sulphuric acid and titrated at 60° with 1% aqueous potassium permanganate; oxalic acid is oxidised rapidly, succinic acid only slowly by this treatment. An excess of sodium chloride was added, the solution was evaporated again, and the residue dried by distillation with benzene and extracted with hot ether. Evaporation of the extract gave crude succinic acid (75 mg.) which after recrystallisation from water gave pure material (25 mg.), m. p. 183—184°, undepressed on admixture with authentic material. The piperazine salt had m. p. 205—206° (decomp.) in agreement with the value given by Pollard, Adelson, and Bain (*loc. cit.*).

Oxidation of the Maleic Anhydride Adduct of Oenanthotoxin Dimethyl Ether.—The crystalline adduct (630 mg.) in purified acetone (100 c.c.) was treated with stirring with portions of powdered potassium permanganate (2·43 g.) during 2 hours. The mixture was then stirred at room temperature for a further hour and then under reflux for ½ hour, cooled, and filtered, and the residue washed first with ice-water, then with hot water. The aqueous washings were united with the residue left by evaporation of the acetone filtrate, treated with 2N-sodium hydroxide, and concentrated to 100 c.c. The solution was acidified and extracted continuously with ether for 16 hours, and the acidic fraction of the extract isolated by successive transfer to sodium hydrogen carbonate solution and to ether. It formed a brown oil which was extracted with cold benzene (3 × 10 c.c.); evaporation of the benzene and distillation of the residue under reduced pressure gave a crude monobasic acid fraction (90 mg.). Chromatography on paper with alcohol–water–ammonia (Long, Quayle, and Stedman, *loc. cit.*) gave a single spot, identical in position with that given by authentic methoxyacetic acid. The crude material was purified by chromatography on powdered cellulose, the same solvent system being used; this gave methoxyacetic acid (24 mg.), identified by conversion into the *benzylamine* salt (30 mg.), plates (from ethyl acetate), m. p. 110—111·5°, undepressed on admixture with a specimen of m. p. 112—113° prepared from the authentic acid (Found : N, 7·3. $C_{10}H_{15}O_3N$ requires N, 7·1%).

Oxidation of the Maleic Anhydride Adduct of Cicutoxin Dimethyl Ether.—The adduct (462 mg.)

was oxidised in acetone (100 c.c.) with potassium permanganate (1·78 g.), and the monobasic acid fraction of the product (68 mg.) isolated in the way used for the adduct from oenanthotoxin dimethyl ether. As before, paper chromatography showed a single spot, identical in position with that given by authentic γ-methoxybutyric acid, but different in position from that given by α-methoxy-*n*-valeric acid. After purification on a column of powdered cellulose γ-methoxybutyric acid (30 mg.) was obtained and characterised as the *piperazine* salt, needles (from acetone), m. p. 101°, undepressed on admixture with a sample of m. p. 102—103° prepared from the authentic acid (Found : N, 8·8. $C_{14}H_{30}O_6N_2$ requires N, 8·7%).

Trideca-6 : 8 : 10-*triene*-2 : 4-*diyne*-1 : 12-*diol*.—Crude penta-2 : 4-diyn-1-ol (2 g.; Bohlmann, *loc. cit.*) in dry benzene (40 c.c.) was added slowly to a stirred solution of ethylmagnesium bromide [from magnesium (1·46 g.), ethyl bromide (4·5 c.c.), and ether (50 c.c.)], and the mixture stirred at 30° for 3 hours. A solution of octatrienal (4·3 g.) in benzene (60 c.c.) was then added slowly, and stirring continued at 30° for 3 hours. The cooled mixture was decomposed by saturated aqueous ammonium chloride (50 c.c.), and the organic layer washed with water, dried, and evaporated. The residue, dissolved in ether (50 c.c.) containing a trace of quinol, was shaken with 5% aqueous sulphuric acid (100 c.c.) at room temperature for 16 hours, and the mixture extracted with benzene. The extract was washed with water, dried, and evaporated. The residue was subjected to systematic chromatography on morin–aluminium oxide as described for the isolation of cicutoxin, the purity of the various fractions being determined by measuring their absorption intensity at 318 and 336 mμ. The purest fractions from the second chromatogram weighed 152 and 225 mg. respectively; they were combined and recrystallised from benzene, giving the pure *diol* (60 mg.) as prisms, m. p. 111—113° (decomp.) (Found : C, 77·4; H, 7·2. $C_{13}H_{14}O_2$ requires C, 77·2; H, 6·9%). On hydrogenation in alcohol with palladium–charcoal, 6·8 mols. of hydrogen were absorbed. Light absorption in EtOH : max. at 243, 252·5, 317·5 and 335 mμ; inflections at 285 and 303 mμ; $10^{-3}\,\varepsilon$, 13·5, 21·3, 57·9, 58·2, 15·0, and 36·0 (see also Fig. 5).

Trideca-3 : 5 : 11-*triene*-7 : 9-*diyn*-2-*ol*.—By reaction with ethylmagnesium bromide (from 0·55 g. of magnesium), hept-2-ene-4 : 6-diyne (1·6 g.) was converted into its Grignard reagent in ether (30 c.c.). The well-stirred solution was treated slowly at 0° during 1 hour with sorbaldehyde (1·8 g.) in ether (20 c.c.), and the mixture heated under reflux for 15 minutes, cooled to 0°, and decomposed with ice and 10% acetic acid. Evaporation of the washed and dried ethereal layer left a residue which was chromatographed on morin–aluminium oxide which had been deactivated by the addition of 4% of water; benzene–light petroleum were used as the solvent; this removed unchanged sorbaldehyde, and elution with methanol and evaporation of the eluate gave the crude reaction product as an oil (1·6 g.) showing the following absorption max. in EtOH : 230, 240, 254, 275·5, and 288 mμ. It was dissolved in 50% aqueous methanol containing 5% concentrated sulphuric acid and a trace of quinol, and after 20 hours at room temperature the solution was diluted and the product isolated with ether. It was purified by chromatography on morin–aluminium oxide, which was controlled by measuring the light-absorption intensity of the fractions at 315 and 336 mμ. Two fractions showing intense absorption at these wave-lengths were obtained. The less strongly adsorbed fraction crystallised from pentane at −50°, giving 2-*methoxytrideca*-3 : 5 : 11-*triene*-7 : 9-*diyne* (30 mg.) as colourless prisms, m. p. *ca.* 0° (Found : C, 83·7; H, 8·2. $C_{14}H_{16}O$ requires C, 84·0; H, 8·0%). Light absorption in EtOH : max. at 214, 250, 266, 279, 296, 315, and 336 mμ; $10^{-3}\,\varepsilon$, 18·3, 31·2, 27·3, 15·8, 27·3, 36·8, and 27·3. With palladium–barium sulphate in alcohol it absorbed 7·1 mols. of hydrogen.

The more strongly adsorbed fraction (200 mg.) was recrystallised from light petroleum, giving *trideca*-3 : 5 : 11-*triene*-7 : 9-*diyn*-2-*ol* (120 mg.) as colourless needles, m. p. 73° (Found : C, 84·2; H, 7·6. $C_{13}H_{14}O$ requires C, 83·9; H, 7·6%). Light absorption in EtOH : max. at 214, 252, 266, 279, 296, 315, and 336 mμ; $10^{-3}\,\varepsilon$, 16·2, 30·8, 27·9, 16·3, 28·5, 39·0, and 28·4 (see also Fig. 4). With palladium–barium sulphate in alcohol it absorbed 7·0 mols. of hydrogen.

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