

191. *Researches on Acetylenic Compounds. Part LIX.* The Synthesis of Three Polyacetylenic Antibiotics.*

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Syntheses of the antibiotics "diatretyne-I," "diatretyne-II," and "agrocybin" are described. In each case a cross-coupling reaction between an appropriate derivative of propiolic acid and a C₆ component was employed.

DIATRETYNE-I was first isolated from culture fluids of the Basidiomycete fungus *Clitocybe diatreta* by Anchel,¹ and was shown² to be a monoamide of an acid of type (I). A sample kindly sent to us was found to absorb intensely at 960 cm.⁻¹, but not at ca. 800 cm.⁻¹; it was



therefore one of the two *trans*-isomers.³ Potentiometric titration gave a p*K*_a value of about 3.55, and reference to data obtained for a range of related acids⁴ established its structure as (II) since, for all αβ-acetylenic acids studied, p*K* values below 2.7 were found.

Synthetical confirmation could most obviously be obtained by a mixed-coupling technique in this instance, employing the ester or amide of propiolic acid as one component and pent-2-en-4-ynoic acid as the other. Methyl propiolate, however, was found by Christensen and Sørensen⁵ to behave abnormally under the usual coupling conditions, giving two products of high oxygen content, the structures of which remain unknown.

trans-Pent-2-en-4-ynoic acid was first shown to couple normally; then the cross-coupling reaction between pentenynoic acid and methyl propiolate was found to give a small yield of the desired half-ester, provided that a low temperature was maintained. Brief treatment with ammonia converted this into a half-amide which showed ultraviolet and infrared spectra identical with those of diatretyne-I and like the latter gave suberic acid on hydrogenation. The biological activity of the synthetic sample, however, was lower than that observed for specimens of the natural compound, a result which was traced to the presence of small amounts of diatretyne-II in existing specimens of the latter.⁶

Diatretyne-II, first isolated from the same cultures as diatretyne-I, was later

* Part LVIII, *J.*, 1957, 4633.

¹ Anchel, *J. Amer. Chem. Soc.*, 1952, **74**, 1588.

² *Idem, ibid.*, 1953, **75**, 4621.

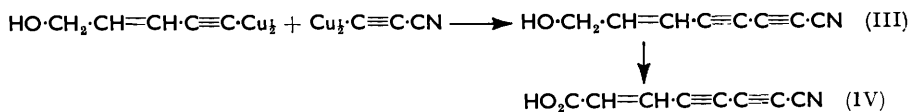
³ Allan, Meakins, and Whiting, *J.*, 1955, 1874.

⁴ Mansfield and Whiting, *J.*, 1956, 4761.

⁵ Christensen and Sørensen, *Acta Chem. Scand.*, 1952, **6**, 893.

⁶ Anchel, personal communication.

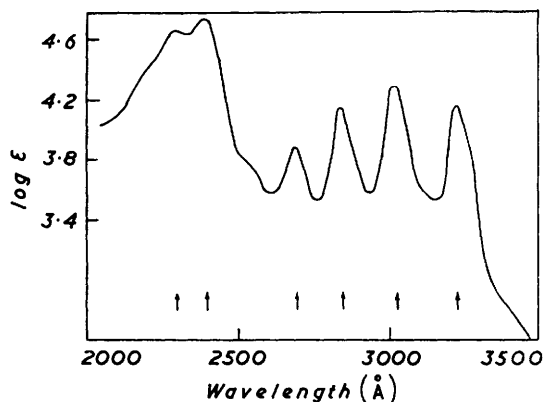
shown to be the corresponding nitrile; ⁷ it was, indeed, obtained in minute yield by the dehydration of diatretyne-I and identified by its biological activity,⁷ in which it far surpasses the amide. For the latter reason an effective synthesis was the more desirable. However, propionitrile failed to give a detectable quantity of cross-coupling products with the acetylenes tested under the usual conditions. The method used by Brockman ⁸ in the direct oxidative coupling of propionitrile, *i.e.*, the action of potassium ferricyanide on the precipitated cuprous derivative, was therefore employed. *trans*-Pent-2-en-4-yn-1-ol was used as the second component, and the desired 7-cyanohept-2-en-4 : 6-diyne-1-ol (III)



was obtained in 14% yield. This proved to be a powerful vesicant (weaker irritant properties are common among acetylenic nitriles). Oxidation with chromic acid and sulphuric acid in acetone gave the corresponding acid, which showed ultraviolet absorption maxima in exact agreement with those recorded for diatretyne-II ^{7*} (Fig. 1).

FIG. 1. *Absorption spectrum of diatretyne-II in alcohol.*

— Synthetic acid (IV). Arrows denote absorption maxima given by Anchel.¹



Agrocybin was first isolated from culture fluids of the Basidiomycete fungus *Agrocybe dura* by Kavanagh, Hervey, and Robbins.⁹ It was subsequently shown to be converted by hydrogenation over platinum into octanamide; and octa-2 : 4 : 6-triynamide was suggested as its structure.¹⁰ This compound was then synthesised;¹¹ the infrared spectrum lacked a few strong bands present in that of the natural compound, but the ultraviolet spectrum showed almost exact agreement. When alkali was added, however, the synthetic amide and the antibiotic reacted, under similar conditions, at quite different rates. In each case the spectrum became more intense and moved to longer wavelengths, but the new product, or products, obtained showed a much sharper fine structure at rather shorter wavelengths in the case of the synthetic amide (Fig. 2). Thus it became clear that

* No intensity data were published for diatretyne-II, and it has not yet been possible to compare the infrared spectrum or the biological activity of the cyano-acid (IV) with those of the antibiotic; proof of identity is thus less complete than for the other polyacetylenic antibiotics. It was, however, possible to prepare from nudic acid B (Florey, Chain, Heatley, Jennings, Sanders, Abraham, and Florey, "Antibiotics," Oxford Univ. Press, 1949, p. 385; Heatley and Stephenson, *Nature*, 1957, **179**, 1078) and from the acid (IV) identical methyl esters (infrared and ultraviolet spectra and mixed melting point), so that the identity of our acid (IV) and nudic acid B is completely established. The spectrum shown in Fig. 1, although superficially similar to those of other polyynes, actually differs significantly from that of any other chromophoric system yet published in the positions of the maxima.

⁷ Anchel, *Science*, 1955, **121**, 607.

⁸ Brockman, *Canad. J. Chem.*, 1955, **33**, 507.

⁹ Kavanagh, Hervey, and Robbins, *Proc. Nat. Acad. Sci. Washington*, 1950, **36**, 102.

¹⁰ Bu'Lock and Jones, *J.*, 1953, 3719.

¹¹ Jones, Thompson, and Whiting, *J.*, 1957, 2012.

agrocybin is not octa-2:4:6-triynamide; and, by elimination, 8-hydroxyocta-2:4:6-triynamide became the most plausible structure. Its synthesis was attempted by cross-coupling methyl propiolate with penta-2:4-diyne-1-ol using cuprous chloride, ammonium chloride, and hydrogen peroxide¹² at low temperatures. The expected hydroxy-ester was obtained as a solid, though only in 5% yield. Its reaction with ammonia to give the amide was virtually complete after a few minutes at -5° ; more normal reaction conditions led apparently to addition, the required amide being lost. The amide showed ultraviolet and infrared spectra identical with those of agrocybin, and reacted with ethanolic sodium

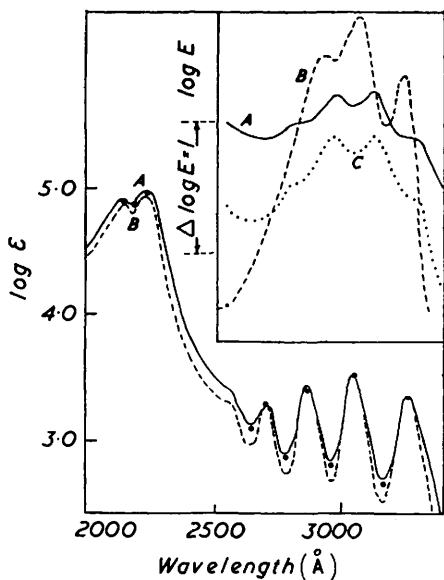


FIG. 2. Absorption spectrum of agrocybin in alcohol.

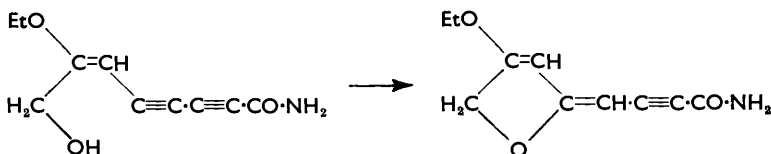
Main diagram: A (—), Synthetic 8-hydroxyocta-2:4:6-triynamide; B (---), synthetic octa-2:4:6-triynamide; C (.), agrocybin (maxima and minima only).

Inset: A, Synthetic 8-hydroxyoctatriynamide; B, synthetic octa-2:4:6-triynoamide; C, agrocybin.

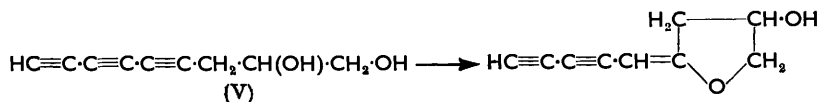
All spectra are for dilute solutions previously treated with a catalytic quantity of sodium hydroxide solution.

(The wavelength-scale applies also to the inset; and although the optical densities are arbitrary, the logarithmic increment is the same in the three spectra and the same as that in the main diagram.)

hydroxide at about the same rate as the latter, giving products with the same absorption spectrum. As a final proof of identity, distribution coefficients between water-ether and water-ethyl acetate were determined.¹³



The difference between the spectra of the ethanol-adducts of 8-hydroxyoctatriynamide and of the simple amide requires explanation. Probably the first product is formed by addition at $\text{C}_{(7)}$ in each case;¹⁴ the agrocybin derivative will then be able to cyclise to a



compound which would be expected to absorb at longer wavelengths, and with less sharp fine structure, than the initial product. Such intramolecular nucleophilic additions take place much more readily than the corresponding intermolecular reactions¹⁵ and are clearly involved in the alkali-catalysed transformation of biformin (V).¹⁶ Superficially the

¹² Milas and Mageli, *J. Amer. Chem. Soc.*, 1953, **75**, 5971.

¹³ Bu'Lock, Jones, Mansfield, Thompson, and Whiting, *Chem. and Ind.*, 1954, 900.

¹⁴ Bohlmann and Viehe, *Chem. Ber.*, 1955, **88**, 1017.

¹⁵ See, *inter al.*, Eglinton, Jones, and Whiting, *J.*, 1952, 2873.

¹⁶ Anchel and Cohen, *J. Biol. Chem.*, 1954, **208**, 319; see Bu'Lock, *Quart. Rev.*, 1956, **10**, 386.

effects, upon absorption spectra, of such processes may resemble those observed in the isomerisations of acetylenic-allenic antibiotics, which have recently been reviewed.¹⁷

Light-absorption maxima for compounds, R·[C≡C]₃·COX, in ethanol.

R = CH ₂ ·OH, X = OMe		R = CH ₂ ·OH, X = OMe		R = CH ₂ ·OH, X = NH ₂		R = CH ₂ ·OH, X = NH ₂	
Å	10 ⁻³ ε	Å	10 ⁻³ ε	Å	10 ⁻³ ε	Å	10 ⁻³ ε
3295	2.1	2260	82	3260	2.25	2700	2.0
3080	3.65	2180	75.5	3045	3.45	2240	100
2890	3.0			2865	2.85	2150	84
2725	1.8						
2580	1.0						

EXPERIMENTAL

Precautions necessary for work with unstable polyacetylenes have been outlined previously;¹⁸ they were needed especially for the triacetylenes related to agrocybin.

Diatretyne-I

trans: *trans-Deca-2:8-diene-4:6-diynedioic Acid*.—*trans*-Pent-2-en-4-ynoic acid (1.65 g.) in acetone (10 c.c.) was added to a solution of cuprous chloride (4 g.) and ammonium chloride (14 g.) in water (20 c.c.). The mixture was stirred in an atmosphere of oxygen until absorption was complete, the temperature being held at 5° by external cooling. Phosphoric acid (20 c.c.; 50%) was added, and the precipitate was collected and extracted with potassium hydroxide solution; reprecipitation and crystallisation from methanol gave the dicarboxylic acid (1.6 g.), which decomposed, when heated, without melting but showed light-absorption properties similar to those recorded by Heilbron *et al.*¹⁹

7-Methoxycarbonylhept-2-ene-4:6-diynoic Acid.—(a) Methyl propiolate (2.0 g.) and pent-2-en-4-ynoic acid (2.0 g.) in acetone (10 c.c.) were added to a mixture of cuprous chloride (10 g.), ammonium chloride (16 g.), and water (30 c.c.), and the solution was stirred in oxygen at *ca.* 5° until absorption was complete (1020 c.c. in 15 hr.). The resultant suspension of basic cupric salts was extracted with ether, without any attempt to filter it, which previous experience has shown to be impracticable and unnecessary. Isolation of the acidic product *via* sodium hydrogen carbonate solution and ether gave a solid which was extracted with boiling light petroleum (b. p. 60—80°); the *half-ester* (0.42 g.) separated from the petroleum on cooling; it had m. p. 134—135° (Found: C, 59.4, 59.8; H, 4.0, 3.5. C₉H₈O₄ requires C, 60.7; H, 3.4%).

(b) The same reactants in 5 c.c. of acetone and 50 c.c. of water were treated with hydrogen peroxide (20 c.c.; "100-vol."), added slowly below the surface of the liquid at 0—10°, during 15 min. Isolation as above gave the *half-ester* (0.37 g.), m. p. and mixed m. p. 134—135°.

Treatment with diazomethane gave the corresponding diester, m. p. 74—75°, which was unexpectedly unstable and gave poor analytical data.

7-Aminocarbonylhept-2-ene-4:6-diynoic Acid (Diatretyne-I).—The above *half-ester* (200 mg.) was added to ammonia solution (*d* 0.88; 5 c.c.) at -5°. After 3 hr. the mixture was cooled to -25°, acidified cautiously with concentrated hydrochloric acid, and extracted with ethyl acetate. Removal of the solvent under reduced pressure left a solid which was extracted with boiling ether; evaporation of the extract and recrystallisation from methanol gave the *half-amide* (80 mg.), decomp. above 195° (Found: C, 58.2, 58.3; H, 3.55, 3.75; N, 8.4. C₈H₆O₃N requires C, 58.9; H, 3.1; N, 8.6%). Light absorption: max. 2230, 2600, 2750, 2900, and 3090 Å (ε 38,000, 12,000, 17,000, 23,000, and 18,500, respectively). A sample of natural origin showed absorption maxima which were identical, within experimental error; Anchel¹ gives 2250, 2600, 2750, 2910 and 3100 Å. Infrared spectra of Nujol suspensions were sufficiently detailed and were identical. On hydrogenation over platinum oxide in ethanol (uptake 92% of the calculated volume), suberic acid, m. p. 144—145°, was formed quantitatively (Anchel¹ gives m. p. 144—145°).

Diatretyne-II

7-Cyanohept-2-ene-4:6-diyn-1-ol.—Cuprous chloride (240 g.) was dissolved in 10% ammonia solution (200 c.c.) under nitrogen, and ammonium chloride (320 g.) and water (2.5 l.) were added.

¹⁷ Bu'Lock, Jones, Leeming, and Thompson, *J.*, 1956, 3767.

¹⁸ Cook, Jones, and Whiting, *J.*, 1952, 2883.

¹⁹ Heilbron, Jones, and Sondheimer, *J.*, 1947, 1586.

A mixture of pent-2-en-4-yn-1-ol (29 g.) and propionitrile (6.0 g.) was run in with stirring, and after 5 min. the yellow precipitate was collected and washed with water until the washings became almost colourless. The wet precipitate was suspended in water (500 c.c.) and stirred briskly while a solution of potassium ferricyanide (320 g.) in water (1 l.) was added during 40 min., the colour changing to dark brown. Ether (300 c.c.) was added, and the stirring was continued for 10 min. The resulting emulsion was centrifuged for 5 min. at 20,000 r.p.m., the ethereal phase was separated, and the aqueous phase and precipitate were stirred with a similar quantity of ether, again separated by centrifugation. After drying (MgSO_4), the ether was distilled off and the residual solid (14 g.) was repeatedly extracted with boiling light petroleum (b. p. 60—80°; 250 c.c. in all). Evaporation gave a solid (3.5 g.) which was crystallised from carbon disulphide, giving the *alcohol* (2.1 g.), m. p. 95—101°, raised by sublimation to 100—101°, with preliminary sintering (Found: C, 72.55; H, 3.9; N, 10.4. $\text{C}_8\text{H}_5\text{ON}$ requires C, 73.25; H, 3.85; N, 10.65%). Deca-2 : 8-diene-4 : 6-diyne-1 : 10-diol was recovered in quantity from the petrol-insoluble residue.

7-Cyanohept-2-ene-4 : 6-diynoic Acid ("Diatretyne II").—The above alcohol (0.5 g.) in acetone (5 c.c.) was treated with a solution of chromic acid (2M in 6M-sulphuric acid; 2.8 c.c.) during 5 min. with external cooling to ca. -15° . After a further 40 min. the mixture was rendered homogeneous by addition of ice-water and extracted with ether (3×30 c.c.). Isolation of the acidic fraction and removal of the solvent under reduced pressure gave the crude acid (300 mg., 50%), m. p. 170—179°. Crystallisation from ether-light petroleum at -70° gave *diatretyne-II* as short needles, m. p. 179—180° (decomp.) (Found: C, 66.2; H, 2.05; N, 9.65. $\text{C}_8\text{H}_5\text{O}_2\text{N}$ requires C, 65.95; H, 2.0; N, 9.5%).

Methyl 7-Cyanohept-2-ene-4 : 6-diynoate (with J. S. STEPHENSON).—(a) The foregoing acid (220 mg.) was treated with 3% sulphuric acid in methanol at 20° for 48 hr. Isolation of the neutral fraction with ether gave the *ester* (230 mg.) as needles, m. p. 102—103.5° (from methylene dichloride-hexane) (Found: C, 68.3; H, 3.0; N, 8.8. $\text{C}_9\text{H}_5\text{O}_2\text{N}$ requires C, 67.9; H, 3.15; N, 8.8%). Light absorption: max. 2315, 2410, 2530, 2685, 2840, 3020, and 3220 Å ($10^{-3}\epsilon$ 42.5, 63, 4.6, 9.1, 18.5, 26.5, and 19.6, respectively).

(b) A sample of nudic acid B (see footnote, p. 951), which had been stored for 10 years at room temperature and had become black (10.5 mg.), was similarly treated with methanolic sulphuric acid. The ester (5 mg.) had m. p. 100—104°, undepressed on admixture with the synthetic sample, and showed identical ultraviolet and infrared absorption spectra (in CCl_4).

Agrocybin

Methyl 8-Hydroxyocta-2 : 4 : 6-triynoate.—To a solution of cuprous chloride (10 g.) and ammonium chloride (16 g.) in water (50 c.c.), penta-2 : 4-diyne-1-ol⁹ (2 g.) and methyl propiolate (2.1 g.) in ethanol (6 c.c.) were added. The mixture was cooled in ice and salt, and 30% hydrogen peroxide (20 c.c.) was added during 1 hr., the temperature remaining below 4°. The mixture was acidified with hydrochloric acid and extracted with ether. The solvent was evaporated, and the residue was chromatographed on deactivated alumina. Evaporation of the benzene eluate gave a residue which solidified and was crystallised four times from methylene chloride at -70° , giving the ester, m. p. 38.5—41.5°. Its structure was confirmed by the spectrographic data above, but it could not be analysed because of the acquisition of static electric charge when disturbed.

8-Hydroxyocta-2 : 4 : 6-triynamide (Agrocybin).—The above ester (110 mg.) was treated with ammonia solution (d 0.88; 2 c.c.) at -5° for 7 min. The solution was then brought to room temperature and extracted with ethyl acetate (40 c.c. in all). After being washed with *N*-sulphuric acid and dried (MgSO_4), the extract was evaporated at 20°. The residue crystallised from tetrahydrofuran-methylene chloride, giving the *amide* (40 mg.) (Found: C, 64.9; H, 3.8; N, 10.8. $\text{C}_8\text{H}_5\text{O}_2\text{N}$ requires C, 65.3; H, 3.4; N, 9.5%).

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