Natural Acetylenes. Part XLVII.[1] Biosynthetic Experiments with the Fungus *Lepista diemii* (Singer). Biogenesis of the C_8 Acetylenic Cyano-acid Diatretyne 2[2]

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The involvement of the crepenynate pathway and an ester of the metabolite $HO{\cdot}CH_2{\cdot}[C{\equiv}C]_3{\cdot}CH{=}^{t}CH{\cdot}CO_2H$ in the biogenesis of $NC{\cdot}[C{\equiv}C]_2{\cdot}CH{=}^{t}CH{\cdot}CO_2H$ (I) has been demonstrated in biosynthetic experiments with *L. diemii* cultures. The necessary ω-oxidation appears to be possible at both the C_{18} and the C_{10} stages. The carbon skeleton of (I) contains C(9)—C(16) of crepenynate.

THE C_8 cyano-acid diatretyne 2 (I; R = H) and the C_{10} hydroxy-acid diatretyne 3 (II; R = H) were found to be the major acetylenic metabolites produced by *Lepista diemii* (Singer), the former predominating in static, the latter in shake cultures.[3] The C_8 carbamoyl acid diatretyne 1 (III; R = H), the commonly found[4] companion of diatretynes 2 and 3, was not detected in this culture even after prolonged growth.

Apart from the cyanogenic glycosides, few naturally occurring nitriles are known, and the combination of

$$NC{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CO_2R \quad (I)$$

$$HO{\cdot}H_2C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CO_2R \quad (II)$$

$$H_2N{\cdot}OC{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CO_2R \quad (III)$$

the polyacetylene and nitrile systems in diatretyne 2

[1] Part XLVI, M. T. W. Hearn, Sir Ewart R. H. Jones, V. Thaller, and J. L. Turner, *J.C.S. Perkin I*, 1974, 2335.

[2] A more detailed account of part of the work described in this paper is in the D.Phil. Thesis of J. L. Turner, Oxford, 1972.

[3] V. Thaller and J. L. Turner, *J.C.S. Perkin I*, 1972, 2032.

[4] M. Anchel, W. B. Silverman, N. Valanju, and C. T. Rogerson, *Mycologia*, 1962, **54**, 249.

made the elucidation of its biogenesis particularly intriguing. We now report the results of biosynthetic studies carried out thus far with *L. diemii*. Since the investigation of the biogenesis of the cyano-acid was the main aim, static cultures were used. In these, the production of the C_8 cyano-acid was satisfactory throughout, but that of the C_{10} hydroxy-acid became erratic towards the end of the experimental runs. The origin of the carbon skeletons and the sequence of reactions leading to the diatretynes were the initial targets of our investigations. The incorporation results obtained are summarised in the Table.

Incorporation of [^{14}C]precursors into diatretynes 2 and 3 [(I) and (II)] by *L. diemii* cultures

				Metabolites					
				(I; R = Me)			(II; R = Me)		
Precursor administered	μCi	Sp. act.†	No. of flasks	mg	Sp. act.‡	% incorp.	mg	Sp. act.‡	% incorp.
$Me[CH_2]_7{\cdot}CH{=}\overset{c}{\underset{*}{C}}H{\cdot}[CH_2]_7{\cdot}CO_2Me$ (IV)	22·4	20	15	82	0·02	0·05	29·5	0·09	0·06
$Me[CH_2]_4{\cdot}CH{=}\overset{c}{C}H{\cdot}CH_2{\cdot}CH{=}\overset{c}{\underset{*}{C}}H{\cdot}[CH_2]_7{\cdot}CO_2Me$ (V)	20	20	24	97	0·03	0·10	49	0·06	0·08
$Me[CH_2]_4{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CH{=}\overset{c}{\underset{*}{C}}H{\cdot}[CH_2]_7{\cdot}CO_2Me$ (VI)	20·2	20	28	81	0·75	1·9	19	1·6	0·8
$\overset{*}{C}H_3{\cdot}[C{\equiv}C]_3{\cdot}CH_2{\cdot}CH{=}\overset{c}{C}H{\cdot}[CH_2]_7{\cdot}CO_2Me$ (VII)	12·3	0·52	30	76	0·008	<0·03	61	6·3	16·4
$Me[C{\equiv}C]_3{\cdot}CH_2{\cdot}CH{=}\overset{c}{\underset{*}{C}}H{\cdot}[CH_2]_7{\cdot}CO_2Me$ (VIII)	10·6	3·1	30	68	2·9	12·5	1·7	6·3	0·5
$HO{\cdot}\overset{*}{C}H_2{\cdot}[C{\equiv}C]_3{\cdot}CH_2{\cdot}CH{=}\overset{c}{C}H{\cdot}[CH_2]_7{\cdot}CO_2Me$ (IX)	25·3	0·38	30	110	0·001	<0·003	67	0·5	0·7
$Me[C{\equiv}C]_3{\cdot}CH{=}\overset{t}{\underset{*}{C}}H{\cdot}CO_2Me$ (X)	8·65	1·05	15	72	2·3	13·2	0·53	72	2·3
$HOCH_2{\cdot}[C{\equiv}C]_3{\cdot}CH{=}\overset{t}{\underset{*}{C}}H{\cdot}CO_2Me$ (II)	8·1	1·05	15	49	0·9	4·0			

† mCi mmol^{-1}. ‡ μCi mmol^{-1}.

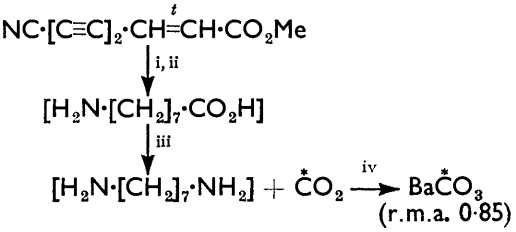
The involvement of the crepenynate pathway [5] in the diatretyne biogenesis was established when methyl oleate (IV), linoleate (V), and crepenynate (VI) were found to be incorporated. The [9-^{14}C][10-^{3}H] C_{18} esters were used as precursors and considerable tritium losses were observed in every case [*cf.* the almost complete tritium retention for the diynetriol (XI) from *Clitocybe rhizophora* [6]]. The purification of metabolites was

$$EtCH(OH){\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH(OH){\cdot}CH_2{\cdot}OH \quad (XI)$$

stopped when constant specific ^{14}C activity was attained; the tritium content was in most instances still decreasing at this stage and cyano-ester samples with 2—35% and hydroxy-ester samples with 6—68% tritium retention were obtained.

The cyano-ester (I; R = Me) from the crepenynate incubation was degraded as shown in Scheme 1; this established that 85% or more of the ^{14}C label was associated with C(1) of the metabolite. Thus, the conversion of crepenynate into the C_8 cyano-acid involves the loss of two (or just possibly three) distal carbon atoms, *i.e.* C(17) and C(18), of the C_{18} precursor chain. This was confirmed when both the [18-^{14}C]triyne ester (VII) and the [9-^{14}C]triyne ester (VIII) were well incorporated into the C_{10} metabolite but none of the [18-^{14}C]ester found its way into the C_8 metabolite. These triyne ester incorporations also proved that the carbon skeleton of the C_{10} hydroxy-acid must consist of C(9)—C(18) of the C_{18} precursors.

trans-[2-^{14}C]Dehydromatricaria ester (X) was incorporated well into both diatretynes, and the C_{10} hydroxy-ester (II; R = Me) was incorporated similarly into the C_8 nitrile, thus suggesting that chain shortening takes place before ω-oxidation and the other transformations at the distal end which lead ultimately to the nitrile group. The ready transformations of [1-^{14}C]-dehydromatricaria ester into related metabolites in experiments with several fungal cultures, in one of which the loss of the ω-carbon atom also occurred, were demonstrated [7] early on and appear to support this



SCHEME 1 Reagents: i, H_2–PtO_2; ii, NaOH–MeOH; iii, NaN_3–H_2SO_4 at 100°; iv, $Ba(OH)_2$

hypothesis. The incorporation of the 18-hydroxy-ester (IX) into the C_{10} hydroxy-acid, though less efficient than with the C_{10} esters (cell wall permeability difficulties could be responsible), indicates that in *L. diemii* cultures hydroxylation can also occur at the C_{18} stage. Alternative pathways might thus be involved in the biogenesis of fungal polyacetylenes (*cf.* the findings with yeast sterols [8]).

The incorporation of the C_{10} hydroxy-ester into the cyano-acid, as well as the consistently higher specific activities obtained for the hydroxy-acid (II; R = H)

[5] J. D. Bu'Lock in 'Comparative Phytochemistry,' ed. T. Swain, Academic Press, London, 1966, p. 79.

[6] G. C. Barley, A. C. Day, U. Graf, Sir Ewart R. H. Jones, I. O'Neill, R. Tachikawa, V. Thaller, and R. A. Vere Hodge, *J. Chem. Soc.* (*C*), 1971, 3308.

[7] P. Hodge, Sir Ewart R. H. Jones, and G. Lowe, *J. Chem. Soc.* (*C*), 1966, 1216.

[8] *E.g.* M. Gryberg, A. C. Oehlschlager, and A. M. Unrau, *J. Amer. Chem. Soc.*, 1973, **95**, 5747.

in all incorporation experiments, do suggest that a C_{10} hydroxy-ester or a closely related precursor with which it is in equilibrium, is on the pathway to the cyano-acid. In incubation experiments with cell-free culture fluid, only ester hydrolysis and no incorporation of dehydromatricaria ester (X) into the diatretynes was observed: this suggests the involvement of intracellular processes in the distal end of chain transformations. The enzymes involved in the $MeC{\equiv}C{\cdot}C{\equiv}C^-$ to $NC{\cdot}C{\equiv}C^-$ transformation must also be fairly specific as no incorporation was found when the labelled C_{11} ester (XII), with its terminal *ethyl* group, was added to the culture in place of dehydromatricaria ester.

$$Et[C{\equiv}C]_3{\cdot}CH{=}\overset{*}{C}H{\cdot}CO_2Me \quad (XII)$$

An attempt to introduce the cyano-group into diatretyne 2 *via* $K^{14}CN$ in a dose harmless to the culture failed (*cf.* the successful incorporation of HCN into asparagine [9]). 3-Indolylacetaldehyde oxime was converted into indole-3-acetonitrile by banana leaf and several fungal species,[10] and oxime-like intermediates might also be involved in the biogenesis of the C_8 cyano-acid. The nitrogen function could be introduced at the C_8 stage [the C_{16} stage (XIII) offered an unlikely alternative] or earlier, with subsequent loss of the carbon atoms from the distal end of the chain. The C_{16} formyl ester (XIII) was not incorporated and orientating experiments with the formyl esters (XIV) and (XV) respectively suggest only the former as a likely precursor.

$$OHC{\cdot}[C{\equiv}C]_2{\cdot}CH_2{\cdot}CH{=}\overset{*}{C}H{\cdot}[CH_2]_7{\cdot}CO_2Me \quad (XIII)$$

$$OHC{\cdot}C{\equiv}C{\cdot}[C{\equiv}C]_2{\cdot}CH{=}\overset{*}{C}H{\cdot}CO_2Me \quad (XIV)$$

$$OHC{\cdot}[C{\equiv}C]_2{\cdot}CH{=}\overset{*}{C}H{\cdot}CO_2Me \quad (XV)$$

The C_{16} and C_8 carbamoyl esters (XVI) and (III; R = Me), respectively, were also tried in incubation experiments, but neither found its way into the cyano-acid. This might indicate that the carbamoyl acid (III; R = H) is formed from the cyano-acid and not *vice versa*, and that the enzymes needed for the nitrile–amide conversion are lacking in *L. diemii*.

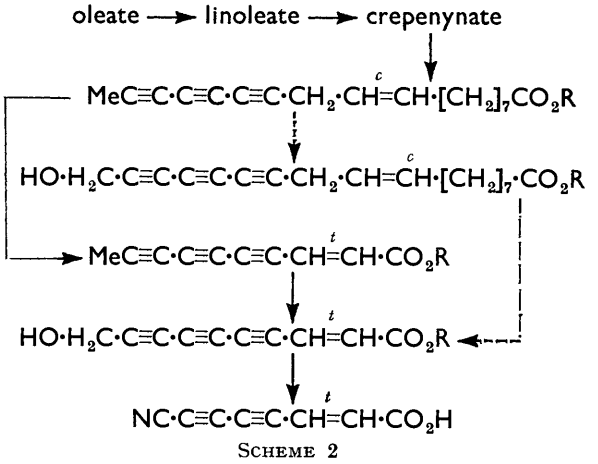
$$H_2N{\cdot}OC{\cdot}[C{\equiv}C]_2{\cdot}CH_2{\cdot}CH{=}CH{\cdot}[CH_2]_7{\cdot}CO_2Me \quad (XVI)$$

The results of the biosynthetic experiments discussed suggest the pathways shown in Scheme 2 for the biosynthesis of the diatretynes. The conversion of further C_{10} precursors into the C_8 cyano-acid is being investigated.

The preparations of the labelled C_{18} and C_{16} esters have been described.[11,12] The C_8, C_{10}, and C_{11} esters were

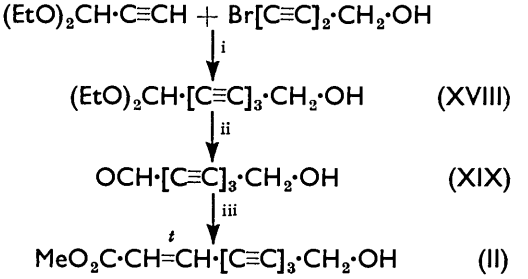
$$Ph_3P{=}\overset{*}{C}H{\cdot}CO_2Me \quad (XVII)$$

labelled at C(2) *via* the phosphorane (XVII).[6] The required *trans*-isomers were formed predominantly in the Wittig reactions and were separated from the *cis*-isomers by chromatography. Dehydromatricaria ester (X) and the homologous C_{11} ester (XII) were thus labelled by the route used before for the synthesis of [1-^{14}C]dehydromatricaria ester;[13] the yield was improved by using dichloromethane instead of benzene in the Wittig reaction.

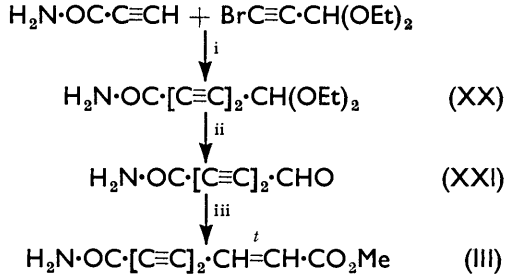


SCHEME 2

The synthesis of the hydroxy-ester (II; R = Me) is outlined in Scheme 3 and that of the carbamoyl ester (III; R = Me) in Scheme 4. In the latter, the coupling



SCHEME 3 Reagents: i, CuCl, $EtNH_2$, $NH_2{\cdot}OH,HCl$; ii, dil. HCl; iii, $Ph_3P{=}CH{\cdot}CO_2Me$, t.l.c.



SCHEME 4 Reagents: i, CuCl, $EtNH_2$, $NH_2{\cdot}OH,HCl$; ii, HCO_2H; iii, $Ph_3P{=}CH{\cdot}CO_2Me$

required similar modifications to that described for the C_{16} amide.[12] The carbamoyl acetal (XX) was stable to hydrochloric acid but hydrolysed smoothly with formic acid.[14] On addition of water to the carbamoyl aldehyde

[9] S. Blumenthal-Goldschmidt, G. W. Butler, and E. E. Conn, *Nature*, 1963, **197**, 718.
[10] S. Mahadevan, *Arch. Biochem. Biophys.*, 1963, **100**, 557.
[11] G. C. Barley, Sir Ewart R. H. Jones, V. Thaller, and R. A. Vere Hodge, *J.C.S. Perkin I*, 1973, 151.
[12] A. G. Fallis, M. T. W. Hearn, Sir Ewart R. H. Jones, V. Thaller, and J. L. Turner, *J.C.S. Perkin I*, 1973, 743.
[13] F. Bohlmann, W. von Kap-herr, C. Rybak, and J. Replinger, *Chem. Ber.*, 1965, **98**, 1736.
[14] A. Gorgues and E. Levas, *Compt. rend.*, 1968, **266C**, 41.

(XXI) an immediate hypsochromic shift was noted in the u.v. spectrum: the resulting trace closely resembled that of the carbamoyl acetal (XX) and the formation of an aldehyde hydrate was suspected. It was highly water-soluble and attempts to isolate it failed. The work-up of the acetal cleavage product had to be carried out in the absence of water and gave low yields of the aldehyde (XXI).

EXPERIMENTAL

For general techniques see Parts XXXIX [11] and XXXIII.[6]

Incubation Experiments.—Surface cultures on malt extract [3] were grown in flasks (15—30) for 40—45 days before the precursors, dissolved in 80% EtOH, were added (1—2 ml per flask). After a further 48—60 h growth the culture fluid was worked up as described.[3] The methylated acid fraction was separated by p.l.c. (petrol–Et_2O, 19 : 1; 4 elutions). The least polar band gave the liquid methyl non-*trans*-2-ene-4,6,8-triynoate.[3] The middle band gave the cyano-ester (I; R = Me), which was recrystallised to constant ^{14}C activity. The most polar band yielded the crude hydroxy-ester (II; R = Me); this was purified by further p.l.c. (petrol–Et_2O, 1 : 1), diluted with inactive hydroxy-ester and crystallised to constant ^{14}C activity.

Data concerning the incubation experiments are given in the Table. [9-^{14}C]- and [10-^{3}H]-labelled [11] oleate (IV), linoleate (V), and crepenynate (VI) were used. In the crepenynate experiment crystallisation to constant tritium activity was attempted: 2% tritium retention was found in the cyano-ester (I; R = Me) and 6% in the hydroxy-ester (II; R = Me). In oleate and linoleate experiments purification was stopped when constant ^{14}C activities were obtained. Tritium data have been omitted from the Table as they do not appear to serve any useful purpose.

The preparation of the remaining labelled esters investigated in the incubation experiments has already been described [12] [(VII), (VIII), (IX), (XIII), and (XVI)] or is given below [(II), (X), and (XII)].

Crepenynate Incubation: Isolation of the Cyano-ester (I; R = Me) *Carboxy-group as Carbon Dioxide.*—The cyano-ester (I; R = Me) (5·76 mg; 0·64 μCi mmol^{-1}), diluted with inactive nitrile (45·32 mg) to a specific activity of 0·072 μCi mmol^{-1}, was dissolved in MeOH (5 ml) and hydrogenated over Adams catalyst (30 mg) at 20° and 756 mmHg for 5 h. The resulting brown oil (50 mg) (no nitrile and amide i.r. absorption discernible) was kept in MeOH (12 ml) with NaOH (0·25 mg) at 20° for 48 h. Acidification (conc. HCl), concentration, and extraction with boiling EtOH (3 × 5 ml) gave on concentration of the extracts a brown solid (53 mg). To this in conc. H_2SO_4 (1 ml), NaN_3 (50 mg) was added at 0° in one portion and the mixture was cautiously heated (100°). The CO_2 liberated during 1 h was trapped as $BaCO_3$; this was dried to constant activity and counted (details of the CO_2 trapping and counting have been described [15]): it had a specific activity of 0·061 μCi mmol^{-1}; relative molar activity (r.m.a.) 0·84.

Methyl 10-*Hydroxydec*-trans-2-*ene*-4,6,8-*triynoate* (II; R = Me) *and Methyl* 10-*Hydroxydec*-cis-2-*ene*-4,6,8-*triynoate.*—3,3-Diethoxypropyne [16] (345 mg, 2·7 mmol) in Et_2O (10 ml)–MeOH (5 ml), containing CuCl (10 mg), NH_2·OH,HCl (100 mg), and $EtNH_2$ (40%; 1·5 ml), and 5-bromopenta-2,4-diyn-1-ol [prepared from penta-2,4-diyn-1-ol [17] (200 mg, 2·5 mmol) and excess of NaOBr in Et_2O–H_2O] gave under the coupling conditions and work-up described [12] a liquid residue (480 mg) which was purified by p.l.c. (petrol–Et_2O, 1 : 1). The zone with R_F 0·3 yielded 1,1-diethoxyocta-2,4,6-triyn-8-ol (XVIII) (160 mg, 31%), $\lambda_{max.}$ (Et_2O) 211 nm (ε 128,000), $\nu_{max.}$ (CCl_4) 3610, 3470, 2215, and 2160 cm^{-1}, τ (CCl_4) 8·79 (6H, t, *J* 7 Hz, C*H*$_3$·CH$_2$), 7·45br (1H, OH), 6·35 (4H, m, CH$_3$·C*H*$_2$·O), 5·69 (2H, s, C*H*$_2$·OH), and 4·77 [1H, s, (EtO)$_2$C*H*]. This (96 mg, 0·47 mmol) was shaken with HCl (2N; 5 ml) for 15 min and the crude hydroxy-aldehyde (XIX) was extracted into Et_2O, and dried ($MgSO_4$–$NaHCO_3$), $\lambda_{max.}$ 336·5, 315, 295, 278, 263, and 227 nm. The Et_2O was replaced by CH_2Cl_2 (10 ml) and the resulting solution was added dropwise to the inactive phosphorane (XVII) [18] (145 mg, 0·43 mmol) stirred in CH_2Cl_2 (4 ml) at −15°. Stirring was continued for 0·5 h at −15° and then for another 0·5 h whilst the mixture was warmed up to 20°. On concentration and p.l.c. (petrol–Et_2O, 2 : 1; 3 elutions) the band with R_F 0·4 yielded needles (32 mg) (CCl_4–petrol) of the *trans*-hydroxy-ester (II; R = Me), m.p. and mixed m.p. 115—116° (decomp.) [lit.,[19] *ca.* 115° (decomp)]. The band with R_F 0·3 gave needles (16 mg) (CS_2) of *methyl* 10-*hydroxydec*-cis-2-*ene*-4,6,8-*triynoate*, m.p. 92—94° (decomp.) (Found: C, 70·5; H, 4·4. $C_{11}H_8O_3$ requires C, 70·2; H, 4·3%), $\lambda_{max.}$ (EtOH) 346 (ε 12,200), 323 (16,300), 303 (11,250), 280 (7500), 256·5 (56,400), and 245·5 nm (46,300), $\nu_{max.}$ (CCl_4) 3620, 3410 (free and bonded OH), 1725 (ester CO), and 1610 cm^{-1} (CH=CH), $\nu_{max.}$ (CS_2) 810 cm^{-1} (*cis*-CH=CH), τ (CCl_4) 6·25 (s, CO$_2$·CH$_3$), 5·68 (s, C*H*$_2$·OH), and 3·75 (AB system, C≡C·C*H*=C*H*·CO$_2$Me), *m/e* 188 (M^+, 100%), 173 (20), 157 (24), 145 (76), 117 (55), 98 (55), 89 (60), and 75 (60); total yield 48 mg (59%), *trans* : *cis* ratio, 2 : 1.

Methyl 10-*Hydroxy*[2-^{14}C]*dec*-trans-2-*ene*-4,6,8-*triynoate* {[2-^{14}C](II; R = Me)}.—The phosphorane (XVII) [6] (37·2 μCi; 11·8 mg; 1·05 mCi mmol^{-1}) and the aldehyde prepared from the acetal (XVIII) (15·0 mg, 0·072 mmol) in CH_2Cl_2 (2 ml) gave the [2-^{14}C]-*cis*-hydroxy-ester (6·87 μCi; 1·05 mCi mmol^{-1}) and the [2-^{14}C]-*trans*-hydroxy-ester (II; R = Me) (16·4 μCi; 1·05 mCi mmol^{-1}); overall yield of ^{14}C 63%, *trans* : *cis* ratio, 2·4 : 1.

Methyl 7-*Carbamoylhept*-trans-2-*ene*-4,6-*diynoate* (III; R = Me) *and Methyl* 7-*Carbamoylhept*-cis-2-*ene*-4,6-*diynoate.*—1-Bromo-3,3-diethoxypropyne [16] (207 mg, 1 mmol) in MeOH (5 ml) was added to CuCl (2 mg), $EtNH_2$ (40%; 0·5 ml), and NH_2·OH,HCl (20 mg), stirred in MeOH–HCO·NMe_2 (1 : 1; 10 ml) under N_2, and was followed by one quarter of a solution of propiolamide [20] (73 mg, 1·05 mmol) in Et_2O (20 ml), more CuCl (20 mg), and the remainder of the propiolamide solution. Usual work-up, p.l.c. (petrol–Et_2O, 1 : 1; 3 elutions), and crystallisation (CCl_4) of the material with R_F 0·35 yielded plates of the *carbamoyl acetal* (XX) (80 mg, 41%), m.p. 57—58° (Found: C, 61·1; H, 6·5; N, 6·9. $C_{10}H_{13}NO_3$ requires C, 61·5;

[15] Sir Ewart R. H. Jones, J. W. Keeping, M. G. Pellatt, and V. Thaller, *J.C.S. Perkin I*, 1973, 148.

[16] J. P. Ward and D. A. van Dorp, *Rec. Trav. chim.*, 1966, **85**, 117; 1967, **86**, 545.

[17] E. R. H. Jones, J. M. Thompson, and M. C. Whiting, *J. Chem. Soc.*, 1957, 2012.

[18] O. Isler, H. Gutmann, M. Montavon, R. Rüegg, G. Rysser, and P. Zeiler, *Helv. Chim. Acta*, 1957, **40**, 1242.

[19] J. N. Gardner, E. R. H. Jones, P. R. Leeming, and J. S. Stephenson, *J. Chem. Soc.*, 1960, 691.

[20] S. Murahashi, T. Takizawa, S. Kurioka, and S. Maekawa, *Nippon Karagaku Zasshi*, 1956, **77**, 1689 (*Chem. Abs.*, 1959, **53**, 5163g).

H, 6·7; N, 7·2%), $\lambda_{max.}$ (EtOH) 271·5 (ε 3000), 256·5 (4700), 242·5 (4700), 230·5 (4800), and 219 nm (4650), $\nu_{max.}$ (CCl_4) 3530, 3480, and 3390 (NH free), 3290 and 3170 (NH bonded), 2240 and 2150 (C≡C), 1695 and 1680 (amide CO), and 1610 cm^{-1} (amide II), τ (CCl_4) 8·8 [t, *J* 7·5 Hz, (CH_3·CH_2·O)$_2$CH], 6·38 [m, (CH_3·CH_2·O)$_2$CH], 4·76 [s, (EtO)$_2$*CH*], and 3·65br and 2·85br (NH_2), *m/e* 195 (*M*$^+$, 0·1%), 150 (100), 140 (12), 123 (11), 105 (11), 94 (18), 77 (30), and 74 (20).

This (250 mg, 1·28 mmol) in HCO_2H (98%, 10 ml) was kept first at 20° for 25 min and then at 50° for 5 min. CH_2Cl_2 (100 ml) was added, the resulting solution of the formyl amide (XXI) ($\lambda_{max.}$ 293, 276, 260, and 245 nm) was further diluted with Et_2O (1800 ml), cooled (0°), and slowly added to the inactive phosphorane (XVII) (470 mg, 1·4 mmol) stirred in CH_2Cl_2 (100 ml) at 0°. The mixture was warmed to 20° over 1 h, washed with aqueous $NaHCO_3$, dried, and concentrated, and the residue was chromatographed on a SiO_2 column (30 g). Elution with petrol–Et_2O (1 : 1; 100 ml fractions) gave (in fractions 8—13) the trans-*carbamoyl ester* (III; R = Me) (152 mg) as yellow prisms ($CHCl_3$), m.p. 127—129° (decomp.) (Found: C, 61·3; H, 3·95; N, 7·6. $C_9H_7NO_3$ requires C, 61·0; H, 4·0; N, 7·9%), $\lambda_{max.}$ (EtOH) 310 (ε 19,500), 291 (23,500), 275 (15,800), 259·5 (10,000), 232 (31,500), and 225 nm (33,000), $\nu_{max.}$ ($CHCl_3$) 3520 and 3400 (NH free), 3340 and 3240 (NH bonded), 2220 and 2140 (C≡C), 1730 (ester CO), 1690 (amide CO), 1620 (CH=CH), and 1587 cm^{-1} (amide II), $\nu_{max.}$ (KBr) 950 cm^{-1} (*trans*-CH=CH), τ (CD_3OD) 6·23 (s, CO_2·CH_3), 3·5 (d, *J* 16 Hz, *trans*-CH=C*H*·CO_2Me), and 3·15 (d, *J* 16 Hz, *trans*-C*H*=CH·CO_2Me), *m/e* 177 (*M*$^+$, 100%), 161 (42), 147 (42), 146 (42), 118 (42), 90 (16), and 74 (55). Fractions 14—18 contained a mixture of the two isomers (12 mg) whilst fractions 19—28 gave *methyl* 7-*carbamoylhept*-cis-2-*ene*-4,6-*diynoate* (18 mg), orange prisms ($CHCl_3$–hexane), m.p. 110—112° (decomp.) (Found: C, 61·1; H, 3·8; N, 7·8. $C_9H_7NO_3$ requires C, 61·0; H, 4·0; N, 7·9%), $\lambda_{max.}$ (EtOH) 313 (ε 13,900), 294 (16,500), 276·5 (11,500), 261 (7800), 234 (27,000), and 227 nm (27,800), $\nu_{max.}$ ($CHCl_3$) 3520 and 3400 (NH free), 3340 (NH bonded), 2140 and 2220 (C≡C), 1730 (ester CO), 1680 (amide CO), 1610 (CH=CH), and 1580 cm^{-1} (amide II), $\nu_{max.}$ (KBr) 810 cm^{-1} (*cis*-CH=CH), τ (CD_3OD) 6·23 (s, CO_2·C*H*$_3$) and 3·66 and 3·55 (each d, *J* 12 Hz, *cis*-CH=CH·CO_2Me), *m/e* 177 (*M*$^+$, 65%), 161 (100), 149 (15), 146 (32), 134 (35), 118 (28), 90 (25), and 74 (76).

Methyl 7-*Carbamoyl*[2-^{14}C]*hept*-trans-2-*ene*-4,6-*diynoate* {[2-^{14}C](III; R = Me)}.—The carbamoyl acetal (XX) (12·5 mg, 0·064 mmol) in HCO_2H (5 ml) was kept at 20° for 0·5 h. CH_2Cl_2 (50 ml) and excess of $NaHCO_3$ were added and the mixture was stirred vigorously for 1 h and filtered. The filtrate was concentrated to 3 ml and added dropwise to the phosphorane (XVII) [6] (37·4 μCi; 12 mg; 1·05 mCi $mmol^{-1}$) stirred in CH_2Cl_2 (2 ml) at −15°. The mixture was warmed to 20° over 0·5 h, concentrated, and purified by p.l.c. (Et_2O; 2 elutions). The band with R_F 0·5 gave the [2-^{14}C]-*trans*-amide {[2-^{14}C](III; R = Me)} (26·1 μCi; 1·05 mCi $mmol^{-1}$) and the band with R_F 0·4 gave the [2-^{14}C]-*cis*-amide (5·1 μCi; 1·05 mCi $mmol^{-1}$). The overall yield of ^{14}C was 82%.

Methyl [2-^{14}C]*Dec*-trans-2-*ene*-4,6,8-*triynoate* (X).—Octa-2,4,6-triyn-1-ol [21] (7·5 mg, 0·063 mmol) in CH_2Cl_2 (20 ml) was shaken with MnO_2 (75 mg) for 0·5 h. The filtrate was concentrated to 10 ml, cooled (−15°), and added dropwise to a solution of the phosphorane (XVII) (14·0 mg, 43·6 μCi; 1·05 mCi $mmol^{-1}$) as described above. T.l.c. purification (petrol–Et_2O, 9 : 1; 2 elutions) gave the *trans*-ester (X) (R_F 0·6; 26·0 μCi; 1·05 mCi $mmol^{-1}$) and its *cis*-isomer (R_F 0·45; 11·78 μCi; 1·05 mCi $mmol^{-1}$), $\lambda_{max.}$ (Et_2O) 345·5 (rel. *E* 2·23), 322·5 (2·95), 302·5 (2·0), 285 (1·0), 255 (10·5), and 244 nm (7·75). The overall yield of ^{14}C was 86%.

Methyl Undec-trans-2-*ene*-4,6,8-*triynoate* (XII) *and Methyl Undec*-cis-2-*ene*-4,6,8-*triynoate*.—The coupling product between penta-2,4-diyn-1-ol (250 mg, 0·1 mmol) and 1-bromobut-1-yne (440 mg, 3·3 mmol) gave on p.l.c. (petrol–Et_2O, 1 : 1) a band with R_F 0·3 from which crude *nona*-2,4,6-*triyn*-1-*ol* (268 mg, 65%) was obtained. It crystallised (CCl_4–petrol) in blades, m.p. 39—40° (Found: C, 81·0; H, 5·8. C_9H_8O requires C, 81·7; H, 6·1%), $\lambda_{max.}$ (EtOH) 311·5 (ε 180), 308 (170), 291·5 (280), 288 (265), 280 (220), 274·5 (265), 270 (60), 259·5 (225), 254·5 (230), and 211 nm (122,000), $\nu_{max.}$ (CCl_4) 3620 and 3500 (OH free and bonded) and 2220 cm^{-1} (C≡C), τ (CCl_4) 8·78 (3H, t, *J* 7 Hz, C*H*$_3$·CH_2), 7·67 (2H, q, *J* 7 Hz, CH_3·C*H*$_2$), 6·75br (1H, OH), and 5·70 (2H, s, C*H*$_2$·OH), *m/e* 132 (*M*$^+$, 100%), 115 (68), 103 (69), 98 (22), 89 (33), 78 (56), 77 (58), and 63 (54).

This (132 mg, 1 mmol) in CH_2Cl_2 (25 ml) with MnO_2 (1·3 g) gave a solution of the corresponding aldehyde, $\lambda_{max.}$ (Et_2O) 339·5 (rel. *E* 2·72), 317 (4·1), 298 (1·85), 266 (1·0), 234 (24·8), and 225 nm (24·8), which was concentrated (5 ml) and treated with the inactive phosphorane (XVII) (334 mg, 1 mmol) in CH_2Cl_2 (5 ml) at −15°. P.l.c. (petrol–Et_2O, 9 : 1; 2 elutions) gave the trans-*ester* (XII) (71 mg) (R_F 0·6), plates (from petrol at −40°), m.p. *ca.* 0°, $\lambda_{max.}$ (EtOH) 345 (ε 16,000), 321·5 (21,500), 301·5 (13,800), 284 (6500), 255·5 (69,500), and 245 nm (51,500), $\nu_{max.}$ (CCl_4) 2220, 2180, and 2110 (C≡C), 1730 (ester CO), 1614 (CH=CH), and 955 cm^{-1} (*trans*-CH=CH), τ (CCl_4) 8·78 (3H, t, *J* 7 Hz, C*H*$_3$·CH_2), 7·64 (2H, q, *J* 7 Hz, CH_3·C*H*$_2$), 6·27 (3H, s, CO_2·CH_3), 3·67 (1H, d, *J* 16 Hz, *trans*-CH=C*H*·CO_2Me), and 3·26 (d, *J* 16 Hz, 1H, *trans*-C*H*=CH·CO_2Me), *m/e* 186 (*M*$^+$, 100%), 171 (40), 155 (30), 143 (55), 127 (25), 115 (55), 98 (24), and 87 (30). The band with R_F 0·5 gave the corresponding cis-*ester* (22 mg), needles (petrol), m.p. 70—72°, $\lambda_{max.}$ (EtOH) 347·5 (ε 12,200), 324 (15,750), 304 (11,100), 286·5 (5400), 256 (70,500), and 246 nm (55,200), $\nu_{max.}$ (CCl_4) 2215, 2180, and 2110 (C≡C), 1730 (ester CO), and 1614 cm^{-1} (CH=CH), $\nu_{max.}$ (CS_2) 812 cm^{-1} (*cis*-CH=CH), τ (CCl_4) 8·78 (3H, t, *J* 7 Hz, C*H*$_3$·CH_2), 7·66 (2H, q, *J* 7 Hz, CH_3·C*H*$_2$), 6·28 (3H, s, CO_2·CH_3), and 3·97 and 3·8 (2H, each d, *J* 11 Hz, *cis*-CH=CH·CO_2Me), *m/e* 186 (*M*$^+$, 100%), 171 (43), 155 (26), 143 (61), 127 (25), 115 (67), 98 (25), and 87 (32).

Methyl [2-^{14}C]*Undec*-trans-2-*ene*-4,6,8-*triynoate* {[2-^{14}C]-(XII)}.—The phosphorane (XVII) (12·4 mg, 39 μCi; specific activity 1·05 mCi $mmol^{-1}$) and nona-2,4,6-triynal [from nonatriynol (7·1 mg, 0·05 mmol)] in CH_2Cl_2 (6 ml) gave the [2-^{14}C]-*trans*-ester {[2-^{14}C](XII)} (20·5 μCi; 1·05 mCi $mmol^{-1}$) and the [2-^{14}C]-*cis*-ester (7·1 μCi; 1·05 mCi $mmol^{-1}$). The overall yield of ^{14}C was 71%.

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[21] W. Chodkiewicz, *Ann. Chim.* (*France*), 1957, **2**, 816.