Patterns of Polyacetylene Production. II. Structural Studies with Some Ten-Carbon Polyacetylenes from *Clitocybe* Species, Using Nuclear Magnetic Resonance and Mass Spectra[1a]

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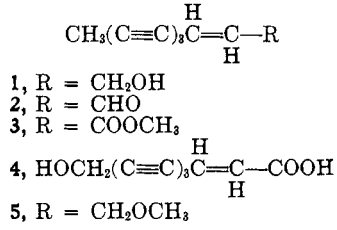
The polyacetylenes of *Clitocybe truncicola* (tentative identification) and *Clitocybe candida* have been examined. A new compound, *trans*-1-methoxy-2-decene-4,6,8-triyne (**5**), has been isolated from *C. candida*. The major polyacetylene from both species is the dehydromatricarianol, *trans*-2-decene-4,6,8-triyn-1-ol (**1**). In addition, *C. truncicola* produces small amounts of the corresponding aldehyde (**2**) and the methyl ester (**3**) of the corresponding acid, as well as a compound which is probably diatretyne-3 (**4**), and trace amounts of an unidentified enediyne and enetriyne. *C. candida* also contains traces of an unidentified enediyne, different from that of *C. truncicola*. These polyacetylenes proved amenable to analysis by mass spectroscopy and gave surprisingly rich spectra with intense molecular ion peaks.

The biological production of polyacetylenes has been examined in recent years from a taxonomic point of view.[2,3] To facilitate this type of study we wished to develop techniques suitable for detection of specific polyacetylenes in minute amounts and to examine the applicability of some of the newer instrumental methods to their identification or structural elucidation. Studies of a group of fungal polyacetylenes, using these techniques and methods in conjunction with more classical ones, are reported in this paper.

Culture liquids of the basidiomycetes *Clitocybe truncicola*[4] and *C. candida*[5] contained similar polyacetylene chromophores. Several polyacetylenes were detected in each species, but the only compound common to both was a dehydromatricarianol, *trans*-2-decene-4,6,8-triyn-1-ol[6,7] (**1**), which was the major component of each. In *C. truncicola* six different polyacetylenes were detected. Three were identified: the alcohol **1**, the corresponding aldehyde, *trans*-2-decene-4,6,8-triynal[7] (**2**), and methyl *trans*-decene-4,6,8-triynoate[8] (**3**), the methyl ester of the corresponding acid. A fourth polyacetylene was identified as diatretyne-3,[9] *trans*-10-hydroxy-2-decene-4,6,8-triynoic acid (**4**). The fifth was partially characterized as an enetriyne more polar than **1**, and the sixth as a still more polar enediyne. From *C. candida*, three polyacetylenes were isolated. One was identified as the alcohol **1**, and a second as the corresponding ether, *trans*-1-methoxy-2-decene-4,6,8-triyne (**5**), a compound not previously reported. The third was characterized as an enediyne. Its structure was not determined.



1, R = CH_2OH
2, R = CHO
3, R = $COOCH_3$
4, $HOCH_2(C{\equiv}C)_3C{=}C(H){-}COOH$
5, R = CH_2OCH_3

Compounds **1**, **2**, **3**, and the unidentified enetriyne were obtained by ethyl acetate extraction of the culture liquids of *C. truncicola* and were separated by countercurrent distribution. The culture liquid was then acidified and extracted again to obtain the compound believed to be **4**. The sixth polyacetylene, a highly polar enediyne, could not be extracted from the culture liquid with organic solvents. It was obtained by adsorption on Norit and elution with aqueous acetone.

Identification of the major polyacetylenic component of *C. truncicola* as I, was based on its ultraviolet and infrared absorption spectra and its melting point, which were in agreement with those previously reported for this compound[7] and on identification of its reduction product as *n*-decanol. Further confirmation was obtained from mass spectrum[10] which indicated a molecular weight of 144, and from nuclear magnetic resonance (n.m.r.) spectrum.[11] The protons of the methyl group attached to acetylene carbon appear as a sharp singlet. The methylene protons appear as a doublet, broadened, apparently by long-range coupling with the β-vinyl proton. The two vinyl protons appear as a broad doublet and a pair of triplets. The pair of triplets is assigned to the α-proton whose signal, due to coupling with the β-proton, would appear as a doublet, which by coupling with the two methylene protons would be further split to triplets. The signal of the β-proton appears as a doublet due to coupling with the α-proton and is broadened by long-range coupling with the methylene protons.

The *C. truncicola* polyacetylene obtained from intermediate tubes of the countercurrent distribution was identified as the aldehyde **2**, on the basis of its ultraviolet spectrum and melting point which, though some-

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(2) N. A. Sörensen, "Chemical Plant Taxonomy," T. Swain, Ed., Academic Press Inc., New York, N. Y., 1963, p. 219.

(3) Part I: M. Anchel, W. B. Silverman, N. Valanju, and C. T. Rogerson, *Mycologia*, **54**, 249 (1962).

(4) This is our strain CR 152, cultured from a fruiting body collected by Dr. C. T. Rogerson in 1962, and tentatively identified as *Clitocybe truncicola*. According to Dr. H. E. Bigelow (University of Massachusetts, Amherst) it may be one of the three species, *Clitocybe truncicola*, *C. marmorea*, or *Pleurotus elongatipes*.

(5) *Clitocybe candida* is our strain H 31 obtained from Dr. R. Heim (Laboratoire de Cryptogamie, Paris).

(6) W. Chodkiewicz, *Ann. chim.* (Paris), [13] **2**, 819 (1957).

(7) J. N. Gardner, E. R. H. Jones, P. R. Leeming, and J. S. Stephenson, *J. Chem. Soc.*, 691 (1960).

(8) J. S. Sörensen, T. Bruun, D. Holme, and N. A. Sörensen, *Acta Chem. Scand.*, **8**, 26 (1954).

(9) M. Anchel, *Arch. Biochem. Biophys.*, **85**, 569 (1959).

(10) Mass spectrum was determined by Dr. P. Funk of Stevens Institute using a CEC 21-103 C mass spectrometer.

(11) The n.m.r. spectrum was determined using a Varian A-60 analytical n.m.r. spectrometer system. We are indebted to Dr. T. R. Stengle for determination of this spectrum and for help in its interpretation.

what low due to impurity, was reasonably close to that previously reported.[7] Additional support for the aldehyde structure **2** was obtained by borohydride reduction. Thin layer chromatography of the product gave a number of spots, one of which had an R_f identical with that of **1**. Further confirmation for the structure was obtained by comparison of the *C. truncicola* aldehyde with a sample of **2** prepared by oxidation of **1**. Both samples were identical in melting point, ultraviolet and infrared spectra, and R_f on a thin layer chromatogram. The 2,4-dinitrophenylhydrazones prepared from both samples likewise proved identical. It is interesting that the dinitrophenylhydrazone shows strikingly enhanced stability as compared to the free aldehyde. This effect may be due in part to the stabilizing effect of bulky end groups, noted by Bohlmann.[12]

Identification of the least polar polyacetylene from *C. truncicola* as the methyl ester **3** was based on similarity of the melting point and the ultraviolet spectrum with those reported for **3**, and was confirmed by infrared, n.m.r.,[13] and mass spectra.[13] The compound **3**, unlike **1** and **2**, has not been isolated previously from fungi, from which only the free acid has been obtained.[7] However, isolation of the ester from higher plants has been reported.[8]

Assignment of structure **4** to the acidic polyacetylene from *C. truncicola* was based on the ultraviolet spectrum of the compound in conjunction with identity of its R_f on a thin layer chromatogram with that of an authentic sample of diatretyne **3** from *Clitocybe diatreta*.[9]

Extracts of *C. candida* culture liquids showed a single enetriyne chromophore. On countercurrent distribution it became apparent that this was due to two separate compounds. The more polar of the two proved identical with the alcohol **1** isolated from *C. truncicola*. The less polar polyacetylene was not identical with any enetriyne reported. The similarity of the ultraviolet absorption spectrum to that of **1** and to that of the ethyl ether of **1**,[14] and the decreased polarity relative to **1**, suggested that it might be a derivative of **1** with a modified hydroxyl group. The infrared spectrum, which was transparent in the carbonyl stretching region ruled out an acyl derivative, leaving an ether as the most likely alternative. Direct demonstration of a methyl ether grouping was obtained by n.m.r. spectrum[13] which showed a sharp peak at τ 6.71.[15a] This evidence strongly suggested the structure **5** for the ether. In confirmation, the remaining peaks of the n.m.r. corresponded to those in the n.m.r. of **1**. Finally, the size of the molecule was established by mass spectrum[13] which indicated a molecular weight of 158. The double bond as in **1** is *trans*, as indicated by a peak in the infrared at 944 cm.$^{-1}$. Thus, although the compound **5** was not isolated in sufficient quantity to allow a determination of empirical formula by classical combustion analysis, the structure of the compound was unequivocally established by physical methods.

A significant development in this work was the demonstration of the feasibility of using mass spectral analysis on polyacetylenes. Although Butler[16] has recently reported on the fragmentation of acetylenic ethers under electron impact, to our knowledge the mass spectra of polyacetylenes have not been recorded previously. To obtain a satisfactory spectrum the compound must give an appreciable concentration of molecules in the vapor phase. For polyacetylenes, the problem is complicated by the competing process of polymerization. Apparently, with our polyacetylenes the tendency to polymerize is not so great as to prevent sufficient vaporization.

For compounds **1**, **2**, **3**, and **5**, spectra were obtained which showed a rich fragmentation pattern. Moreover, they showed intense molecular ion peaks, consistent with the stabilizing influence of an extended π-electron system.[17] The feasibility of using mass spectroscopy for determination of molecular weight of polyacetylenes is especially gratifying since it could not be confidently predicted.

In addition to practical advantages, mass spectra of polyacetylenes offer intriguing possibilities for theoretical interpretation. The fragmentation pattern consists of groups of peaks corresponding to units containing five to nine carbons. The members within each group differ by the mass of a single hydrogen. Each fragment with a given number of carbons bears hydrogens ranging in number from one to at least five. The larger carbon units may have up to eight hydrogens. This fragmentation pattern seems best interpreted as arising by migration of hydrogens along the carbon chain of the molecular ion followed by cleavage.

We plan to report in more detail later, on the mass spectra of compounds **1**, **2**, **3**, and **5**, as well as to extend the study to other polyacetylenes, in order to determine the generality of this type of fragmentation.

Experimental

Melting points were taken on a hot stage[18] equipped with a polarizing microscope, and are corrected. The infrared spectra were determined on KBr pellets with a Perkin-Elmer Model 21 spectrophotometer. Ultraviolet spectra were run on a Cary Model 11 UV-VIS spectrophotometer. Removal of solvents was carried out under reduced pressure (water pump) at temperatures not exceeding 50°, and in the final stages of concentration, under a stream of nitrogen in dim light. Thin layer chromatography (t.l.c.) was run using as adsorbent, silica gel G (manufactured by E. Merck, A. G., Darmstadt, Germany; distributed by Brinkmann Instruments, Inc., Great Neck, N. Y.). Plates were activated by heating at 110° for 0.5 hr., and stored in a desiccator. They were spotted and developed in dim light. Developed plates were visualized wth iodine vapor. Since the crystalline polyacetylenes are unstable, the compounds were stored in solution at 5°. Countercurrent distribution was carried out either in a 50-tube apparatus with 75-ml. phases, or in a 60-tube apparatus with 10-ml. phases.[18]

The Polyacetylenes of *C. truncicola*.—In a typical run, *C. truncicola* was grown in the dark at 20° on a reciprocal shaker, in 500-ml. erlenmeyer flasks, each containing 130 ml. of peptone

(12) F. Bohlmann, *Chem. Ber.*, **86**, 657 (1953).

(13) N.m.r. spectra were carried out by Mr. W. T. Lewis of Mobil Chemical Co. on a carbon tetrachloride solution, using tetramethylsilane as reference. Mass spectra were carried out by Mr. J. Bendoraitis of Socony Mobil Oil Co., Inc. (Paulsboro, N. J.) using a CEC 21-103 (modified) mass spectrometer. We are indebted to Dr. D. D. Phillips through whose courtesy these spectra were obtained.

(14) F. Bohlmann and H. G. Viehe, *Chem. Ber.*, **87**, 712 (1954).

(15) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Inc., New York, N. Y., 1959: (a) p. 65; (b) p. 60; (c) p. 61.

(16) P. E. Butler, *J. Org. Chem.*, **29**, 3024 (1964).

(17) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 51.

(18) The melting point hot stage and the countercurrent distribution apparatus were manufactured by Otto Post, Maspeth, N. Y.

broth.[19a] The production of polyacetylenes, which was followed spectrophotometrically by measuring the intensity of an ultraviolet absorption peak at 330 mμ, reached a maximum about 30 days after inoculation, when the cultures were harvested by filtering through cheesecloth to remove the mycelium. A total of 5.8 l. of culture liquid was obtained which was extracted first with 1250 ml., then 650 ml. of ethyl acetate to give a polyacetylene fraction A. The culture medium was then acidified with dilute hydrochloric acid to pH 2 and extracted with two 600-ml. portions of ethyl acetate to give an acidic polyacetylene fraction B. The water layer still showed weak ultraviolet absorption typical of a polyacetylene, with longest wave length at 280 mμ. This material was adsorbed on charcoal by stirring the culture liquid with 120 g. of Norit A (Pfanstiehl) for 1.5 hr. After filtration the charcoal was washed twice with 240-ml. portions of water and then extracted successively with 575-, 275-, and 275-ml. portions of an acetone–water solution (8:2 v./v.), each portion being stirred with the charcoal for 0.5 hr. The combined eluates (fraction C) were concentrated to a volume of 220 ml., giving a solution which showed peaks in the ultraviolet at 250, 265, and 280 mμ. The total amount of this enediyne in C was estimated spectrophotometrically to be about 0.6 mg. (based on extinction coefficients of an enediyne chromophore[20] and assuming a molecular weight of 200). Lack of material prevented further identification.

The ethyl acetate solution containing the acidic polyacetylene fraction B had an ultraviolet spectrum with peaks at 280, 300, 318, and 340 mμ. The total amount present was 5 mg. (spectrophotometrically estimated on the basis of extinction coefficients of diatretyne-3[9]). Thin layer chromatography using ethyl acetate–acetic acid (50:1 v./v.) as developer showed several spots. The most intense spot had the same R_f as an authentic sample of diatretyne-3 from *C. dialreta*. Lack of material prevented a more positive identification.

Polyacetylene Fraction A.—The ethyl acetate extract of the unacidified culture liquid was concentrated to a volume of about 30 ml. The remainder of the ethyl acetate was carefully evaporated over 20 ml. of water. The residue, a mixture of oil and crystals suspended in water, was dissolved by addition of 40 ml. of methanol, and the solution was subjected to countercurrent distribution between Skellysolve B[21] and a 2:1 solution of methanol–water for 50 transfers. Spectrophotometric analysis showed that tubes 0–8 contained an enetriyne with maximum concentration in tube 3. This polyacetylene was identified as *trans*-2-decene-4,6,8-triyn-1-ol (**1**). The distribution curve showed a disproportionately high concentration of enetriyne in tube 0, indicating the presence of a second, more polar enetriyne that does not move in the solvent system used. The total amount of this unknown compound was about 15 mg. (estimated spectrophotometrically). This enetriyne has not been further characterized. Tubes 24–38 contained *trans*-2-decene-4,6,8-triynal (**2**) with maximum concentration in tube 32. Tubes 41–49 contained methyl *trans*-2-decene-4,6,8-triynoate (**3**) with maximum concentration in tube 46. Tubes 39 and 40 were mixtures of **2** and **3**.

***trans*-2-Decene-4,6,8-triyn-1-ol (Dehydromatricarianol, 1).**—Tubes 2–6 were combined. The upper phase was removed in the presence of the lower phase and the latter was concentrated until crystals started to appear. An equal volume of water was added and the mixture was extracted with two 40-ml. portions of ethyl acetate. The total amount of enetriyne present was estimated spectrophotometrically to be about 215 mg. The ethyl acetate solution was evaporated to dryness and the residue was recrystallized from aqueous methanol to give 173 mg. of product. Further recrystallization of a portion of the material gave lath-like crystals, m.p. 129–130° (lit.[20] m.p. 128–129°). The ultraviolet data [λ_{max}^{MeOH} 229 mμ (log ε 4.96), 240 (5.17), 257 (3.62), 272 (3.95), 288 (4.22), 307 (4.33), and 328 (4.17)] and infrared data [ν_{max} 3330 (OH), 2200 (C≡C), 952, and 940 cm.$^{-1}$ (*trans* CH=CH)] agree with those reported for **1**.[7] Hydrogenation in ethyl acetate at atmospheric pressure using a 10% palladium on calcium carbonate catalyst gave an oily product which was converted by Brewster's method[22] to the 3,5-dinitrobenzoate derivative. This melted at 55–56° (lit.[23a] m.p. 57.7°), and the melting point was not depressed on mixture with an authentic sample. The n.m.r.[11] (in deuteriochloroform) had peaks at τ 3.2, 3.6 (pair of triplets, α-vinyl H), 4.0, 4.3 (broad doublet, β-vinyl H), 5.7 (broad doublet, CH_2), and 8.0 (sharp singlet, C≡C—CH_3). Mass spectrum[10] showed a molecular ion with *m/e* 144.

***trans*-2-Decene-4,6,8-triynal (2).**—The combined top layers from tubes 28–37 were concentrated to dryness to give 1.2 mg. of crude product. Recrystallization from Skellysolve B gave yellow crystals, sintering at 80°, melting at 98–103° (lit.[7] m.p. 108–109°). The ultraviolet absorption spectrum (in cyclohexane) agreed with that reported, except for ε values, which were about 20% too low. A "synthetic" sample of **2** was prepared by oxidation of **I** with manganese dioxide.[7] The infrared spectra of the natural and "synthetic" samples were identical. The natural aldehyde and "synthetic" material showed identical behavior on a thin layer chromatogram. With Skellysolve B–ethyl acetate (3:1 v./v.) as developer, the R_f was 0.62. The mass spectrum of the synthetic aldehyde showed a molecular ion with *m/e* 142.[10]

A tiny sample (<1 mg.) of the natural aldehyde in methanol solution was allowed to react with sodium borohydride overnight at room temperature. The reaction mixture was diluted with 2 vol. of water and extracted twice with ethyl acetate. The extracts were dried with anhydrous sodium sulfate and concentrated to a very small volume. Examination of this concentrate of the crude reaction mixture by t.l.c. (developer Skellysolve B–ethyl acetate, 3:1 v./v.) showed a mixture of several compounds, one of which was identical with **1** in t.l.c. behavior. The reaction mixture was not examined further.

***trans*-2-Decene-4,6,8-triynal 2,4-Dinitrophenylhydrazone.**—Addition of 1.5 ml. of a freshly prepared 2,4-dinitrophenylhydrazine solution[23b] to 48 mg. of "synthetic" *trans*-2-decene-4,6,8-triynal (**2**) in 2 ml. of methanol gave an immediate red precipitate which after standing for 0.5 hr. in the cold was collected by filtration and recrystallized from ethyl acetate. A yield of 40 mg. (37%) of chunky, blood-red crystals was obtained. Unlike the parent compound, the derivative was moderately stable and could be stored in the cold as crystals for at least a day without apparent decomposition. This 2,4-dinitrophenylhydrazone and the one obtained from the natural aldehyde had the same melting point and ultraviolet and infrared spectra. They showed no sharp melting point but from 180–200° gradually turned to a steely blue-black color without other indication of decomposition: λ_{max}^{MeOH} 243 mμ (log ε 4.44), 270 infl. (4.16), 285 infl. (3.96), 312 (3.92), 334 infl. (4.03), and 406 (4.60); ν_{max} 2220, 2160, 2090 (C≡C), 16.10 (C=N), 1505 (C—NO_2), 1330 (Ar—N), 1133 (Ar—H), 964 (*trans*-CH=CH), and 830 cm.$^{-1}$ (Ar—H).

Anal.[24] Calcd. for $C_{16}H_{10}N_4O_4$: C, 59.63; H, 3.13; N, 17.39; O, 19.86. Found: C, 59.68, 59.88; H, 3.32, 3.32; N, 17.10; O, 19.59.

Methyl *trans*-2-Decene-4,6,8-triynoate (3).—The total amount of **3** present in tubes 43–48 (estimated spectrophotometrically) was about 6 mg. For isolation, the combined top layers of these tubes were concentrated nearly to dryness. Upon cooling in Dry Ice, crystals formed, m.p. 99–106°. Recrystallization by slow evaporation of a Skellysolve B solution gave crystals: m.p. 107–108° (lit.[7] m.p. 105–106°); λ_{max}^{MeOH} 243 mμ (log ε 4.59) 254 (4.70), 284 (3.75), 301 (4.04), 322 (4.20), and 344 (4.60); ν_{max} 2910 (C—H), 2220, 2170, 2100 (C≡C), 1715 (ester C=O), 1610 (C=C), and 960 (*trans* CH=CH). The n.m.r.[13] (in carbon tetrachloride) showed peaks at τ 3.40 (O=C(H)—CH=),[15c] 3.60 (=CH—C≡C—),[25] 6.29 (—O—CH_3),[25] and 7.93 cm.$^{-1}$ (—C≡C—CH_3).[25] Mass spectrum[13] showed a molecular ion with *m/e* of 172.

(19) (a) The peptone broth contained per liter 10 g. of dextrose, 1.5 g. of KH_2PO_4, 0.5 g. of $MgSO_4 \cdot 7H_2O$, 1.0 g. of neopeptone, 0.6 μmole of thiamine, and 0.5 ml. of a mixture of minor mineral elements as used in this laboratory: H. M. Yusef, *Bull. Torrey Botan. Club*, **80**, 43 (1953). (b) Cornsteep medium contains per liter: 1.5 g. of KH_2PO_4, 0.5 g. of KCl, 0.5 g. of $MgSO_4 \cdot 7H_2O$, 3 g. of $NaNO_3$, 40 g. of dextrose, and 5 g. of Staley special nutrient 22 (cornsteep, obtained from A. E. Staley Manufacturing Co., Decatur, Ill.).

(20) E. R. H. Jones, M. C. Whiting, J. B. Armitage, C. L. Cook, and N. Entwistle, *Nature*, **160**, 900 (1951).

(21) Skellysolve B is essentially a normal hexane fraction (b.p. 60–70°) obtained from Skelly Oil Co., Kansas City 10, Mo.

(22) J. H. Brewster and C. J. Ciotti, Jr., *J. Am. Chem. Soc.*, **77**, 6214 (1955).

(23) R. L. Shriner, R. C. Fuson, and D. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y.: (a) p. 281; (b) p. 219.

(24) Microanalyses were by Schwartzkopf Microanalytical Laboratory, Woodside, N. Y.

(25) F. Bohlmann, C. Arndt, H. Bornowski, and K. Kleine, *Chem. Ber.*, **96**, 1485 (1963).

Polyacetylenes of *Clitocybe candida*.—Shake cultures of *C. candida* were grown in cornsteep medium[19b] in the dark at 25°, either in 2-l. flasks containing 520 ml. of culture medium, or in 1-l. flasks containing 260 ml. of culture medium. Production of polyacetylenes was followed spectrophotometrically. The concentration of polyacetylenes slowly increased for about 4 weeks and showed little change thereafter. The mycelium was removed by filtration through cheesecloth and the culture liquid was extracted successively with 1400-, 700, and 700-ml. portions of ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and concentrated to a volume of about 25 ml. The remainder of the ethyl acetate was removed over 20 ml. of water. To this was added 40 ml. of methanol and the resulting solution was distributed between Skellysolve B and a 2:1 methanol–water solution for 50 transfers, using the first two tubes for the initial sample. Spectrophotometric analysis showed the presence of an enetriyne in tubes 0–9 with peak concentration in tube 3 and a second enetriyne in tubes 35–48 with peak concentration in tube 43.

***trans*-2-Decene-4,6,8-triyn-1-ol (1).**—Tubes 2–9 from the countercurrent distribution were combined, the lower layer was separated and concentrated to a volume of about 170 ml., and the resulting aqueous solution was extracted first with the top layer from tubes 2–9, then with two 100-ml. portions of fresh Skellysolve B. The combined Skellysolve extracts were concentrated to a volume of 15 ml. and the mixture was distributed between Skellysolve B and 1:2 methanol–water solution for 60 transfers. Spectrophotometric analysis showed the polyacetylene in tubes 25–40 with maximum concentration in tube 32. The total amount (estimated spectrophotometrically) was about 25 mg. Tubes 25–40 were combined. The lower layer was separated and concentrated to about half its initial volume, then extracted with two 100-ml. portions of benzene. The extract was concentrated to a small volume, combined with the top layer from the countercurrent distribution, and concentrated to about 0.5 ml. Thin layer chromatography (developer Skellysolve B–ethyl acetate, 1:1 v./v.) indicated that the concentrate was a mixture of about seven or eight components. The polyacetylene was separated by preparative t.l.c. in which the developed chromatogram was divided into seven portions using a previously run chromatogram as a guide and extracting each portion with methanol. The extracts with polyacetylene ultraviolet spectra were combined (R_f approximately 0.52 for a Skellysolve B–ethyl acetate 1:1 developer) and evaporated to dryness, giving a semicrystalline residue. After several recrystallizations from benzene–Skellysolve B and finally from methanol–water a few milligrams of crystalline product was obtained, identical with **1** isolated from *C. truncicola* in melting point, ultraviolet and infrared spectra, and t.l.c. behavior.

In the preparative thin layer chromatogram an extract was obtained of a compound with higher R_f (0.71) that had an ultraviolet spectrum with peaks at 252, 265, and 283 mμ, typical of an enediyne. However, there was too little material for identification.

***trans*-1-Methoxy-2-decene-4,6,8-triyne (5).**—Tubes 38–48 from the countercurrent distribution of the initial extract of the culture liquid of *C. candida* were combined, the layers were separated, and the lower layer was concentrated, then extracted with two 75-ml. portions of Skellysolve B. The combined upper layer and Skellysolve B extracts were concentrated to a volume of about 5 ml. and the mixture was distributed between Skellysolve B and a 9:1 methanol–water solution for 60 transfers. The polyacetylene appeared in tubes 23–32, with highest concentration in tube 28. These tubes were combined, the lower layer was separated, and most of the methanol was removed. The aqueous concentrate was extracted with Skellysolve B four times. The extracts were combined with the original top layer, dried over anhydrous sodium sulfate, and concentrated. A thin layer chromatogram of the concentrate using Skellysolve B–ethyl acetate (4:1 v./v.) as developer showed two spots, of which the upper (R_f 0.60) was the polyacetylene. Preparative t.l.c. followed by methanol extraction of the appropriate regions of the chromatogram gave a solution from which a small amount of semisolid material was obtained. This was crystallized from Skellysolve B. The amount of sample available was insufficient for elemental analysis. The compound sintered at 34° and melted at 40–40.5°: λ_{max}^{MeOH} 229 mμ (log ε 4.88), 240 (5.09), 257 (3.62), 271 (3.93), 288 (4.20), 307 (4.29), and 328 (4.14); ν_{max} 2900 (C—H), 2220, 2190 (C≡C), 1448 (OCH_3), and 944 cm.$^{-1}$ (*trans*-CH=CH). The n.m.r. spectrum (in carbon tetrachloride) showed peaks[15,25] at τ 3.5–3.8 (a region of weak peaks obscured by background, α-vinyl H), 4.2 (broad peak, β-vinyl H), 6.05, 6.07, 6.14, and 6.17 (CH_2), 6.71 (O—CH_3), and 8.04 (C≡C—CH_3). Mass spectrum[13] showed a molecular ion with *m/e* 158.

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The Benzohydroxamate Anion

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The benzohydroxamate anion exists in aqueous solution in forms $RCONHO^-$ (A) and $RC(=O)N\bar{O}H \longleftrightarrow RC(-\bar{O})=NOH$ (B) in approximately equal concentration. The possible existence of a third form, $RC(-OH=NO^-$ (C), cannot be disproved; however, if it does exist, its maximum concentration would be not more than 10^{-3} to 10^{-4} times that of the other species.

The hydroxamate anion is a particularly effective nucleophile for attack on the phosphorus atom in phosphoric and phosphonic anhydrides and halides.[1,2] In aqueous solution, the monoanion can potentially exist in three forms in equilibrium with two protonated forms as indicated in Scheme I. From the analysis of ultraviolet absorption spectra Plapinger[3] concluded that in aqueous solution the anion exists in at least two, and possibly all, of the three forms, and that one of the forms is certainly A. In addition, the spectral evidence pointed to the existence of M as the predominant protonated form. Infrared spectra of hydroxamic acids in solvents of graded polarity (but not including water) give strong support to this conclusion.[4–7]

In early reports of the reaction between hydroxamate anions and phosphonofluoridates[2,8,9] it was suggested

(1) B. E. Hackley, Jr., R. Plapinger, M. Stolberg, and T. Wagner-Jauregg, *J. Am. Chem. Soc.*, **77**, 3651 (1955).

(2) R. Swidler and G. M. Steinberg, *ibid.*, **78**, 3594 (1956).

(3) R. E. Plapinger, *J. Org. Chem.*, **24**, 802 (1959).

(4) E. M. Usova and E. M. Voroshia, *Proc. Acad. Sci. USSR, Chem. Sect.*, **113**, 425 (1957).

(5) D. Hadzi and D. Prevorsek, *Spectrochim. Acta*, **10**, 38 (1957).

(6) From infrared spectra, Exner[7] has recently concluded that in dioxane and chloroform solutions and in the crystalline state the benzohydroxamate anion exists in form B.

(7) O. Exner, *Collection Czech. Chem. Commun.*, **28**, 1656 (1964); **29**, 1337 (1964).

(8) T. Wagner-Jauregg, *Arzneimittel-Forsch.*, **6**, 194 (1956).

(9) M. A. Stolberg and W. A. Mosher, *J. Am. Chem. Soc.*, **79**, 2618 (1957).