Chem. Ber. 114, 468 – 476 (1981)

Isolation and Structural Elucidation of *Oenanthe aquatica* (L.) Fruit C_{15} Polyacetylene Hydrocarbons

Franco F. Vincieri, Silvia A. Coran, Valerio Giannellini,* and *Massimo Bambagiotti A.*

Istituto di Chimica Farmaceutica e Tossicologica dell'Università,
Via G. Capponi, 9, I-50121 Firenze, Italy

Received May 28, 1980

From the fruits of *Oenanthe aquatica* (L.), a plant used in the past in popular medicine, a group of C_{15} polyacetylene hydrocarbons have been isolated and structurally identified on the basis of spectroscopic evidence. 2-*trans*-9-*cis*-2,9-pentadecadiene-4,6-diyne (**1**), 2-*trans*-8-*cis*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne (**2**), and 2-*trans*-8-*trans*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne (**3**) are prominent, while the two other geometrical isomers of **2** and **3** (**4** and **5**) exist in small amounts. The results support the generally accepted idea of a parallelism between the biosynthetic pathways of the C_{15} and the much more widespread C_{17} polyacetylenes.

Isolierung und Konfigurationsermittlung von C_{15}-Polyacetylen-Kohlenwasserstoffen aus den Früchten von *Oenanthe aquatica* (L.)

Aus den früher in der Volksmedizin verwendeten Früchten von *Oenanthe aquatica* (L.) wurde eine Gruppe von C_{15}-Polyacetylen-Kohlenwasserstoffen isoliert. Ihre Konfigurationen wurden auf Grund spektroskopischer Daten ermittelt. 2-*trans*-9-*cis*-2,9-Pentadecadien-4,6-diin (**1**), 2-*trans*-8-*cis*-10-*trans*-2,8,10-Pentadecatrien-4,6-diin (**2**) und 2-*trans*-8-*trans*-10-*trans*-2,8,10-Pentadecatrien-4,6-diin (**3**) überwiegen, während die beiden anderen geometrischen Isomeren von **2** und **3** (**4** und **5**) in geringer Menge vorkommen. Die gefundenen Daten bestätigen die allgemein akzeptierte Meinung, daß die Biosyntheseschritte von C_{15}- und den weiterverbreiteten C_{17}-Polyacetylenen gleich sind.

In a previous study undertaken to investigate the pharmacological properties of *Oenanthe aquatica* fruit[1], already known in the past for its medicinal characteristics, we detected a group of highly unsaturated hydrocarbons which appeared as final peaks on the gas chromatogram of the high boiling hydrocarbon fraction of the oil. Only the most abundant components of the group were isolated in very small amounts by preparative gas chromatography and recognized as belonging to the C_{15} polyacetylene class on the basis of their characteristic UV spectra and MS measurements. In particular for one compound (**1**) the chromophore involved and the M.W. of 200 pointed out a $R-=-\equiv-\equiv-R'$ polyacetylene structure, with an additional unconjugated double bond in R or R', while the M. W. of 198 and the chromophore of both the remaining most prominent compounds (**2** and **3**) strongly suggested an $R''-=-\equiv-\equiv-=-=-=-R'''$ C_{15} structure with R'' and R''' as alkyl substituents. In the latter case the collected data indicated that the structural differences between **2** and **3** might only reside in some geometrical isomerism of double bonds.

0009 – 2940/81/0202 – 0468 $ 02.50/0

These findings were sufficient to recognize that we were faced with a group of C_{15} polyynes giving us a good chance of investigating the biogenesis of this rare class of naturally occurring compounds.

With this aim, the presence of these substances in the fruit of *Oenanthe aquatica* has been thoroughly reconsidered in order to obtain full structural information on all occurring polyacetylene hydrocarbons, even those detectable in very small amounts.

Results and Discussion

Whereas the biosynthetic pathways of the C_{17} polyacetylenes have been elucidated by the discovery in nature of a considerable number of the proposed precursors and members of the class, the same has not happened for the much less widespread C_{15} polyacetylenes. However, it is known that the C_{15} polyacetylenes may be related biosynthetically to the C_{17} polyacetylenes essentially by key structural features indicating a common origin from oleic acid. One of these is the position of the isolated *cis*-double bond of many C_{15} and C_{17} acetylenes which is the same as in oleic acid, if we count the carbon atoms of the acid beginning from the "distal" part of its molecule, i. e. the end furthest from the carboxyl group[2a]. In the triglycerides of the *Oenanthe aquatica* fruit we found a large predominance of oleic acid, with linoleic acid in substantial proportion and palmitic and stearic acid in very small amounts.

In the case of C_{17} polyynes the proposed biosynthetic pathway, starting with oleic acid, proceeds *via* dehydrogenation, oxidation, and shortening steps leading to 2-*trans*-9-*cis*-2,9-heptadecadiene-4,6-diyne with an isolated double bond in the same position as in oleic acid. Therefore, it may be regarded as one of the most biosynthetically significant C_{17} polyyne hydrocarbons. This compound has been observed among the polyacetylenes isolated from the roots of *Oenanthe crocata*[3]. The same biogenetic route involving a further β-oxidation to reduce the chain length has been proposed in the case of C_{15} polyynes[2b]. In this view, 2-*trans*-9-*cis*-2,9-pentadecadiene-4,6-diyne (**1**) could assume the same significance as its above-mentioned C_{17} homolog but has been merely postulated in the biosynthetic schemes[2c]. However, this structure was assigned to a not completely pure sample of a hydrocarbon obtained from the roots of *Pittosporum buchanami*[4].

For unambiguous identification of the proposed structure, the isolation of **1** in high purity became mandatory to obtain good quality NMR and IR spectra.

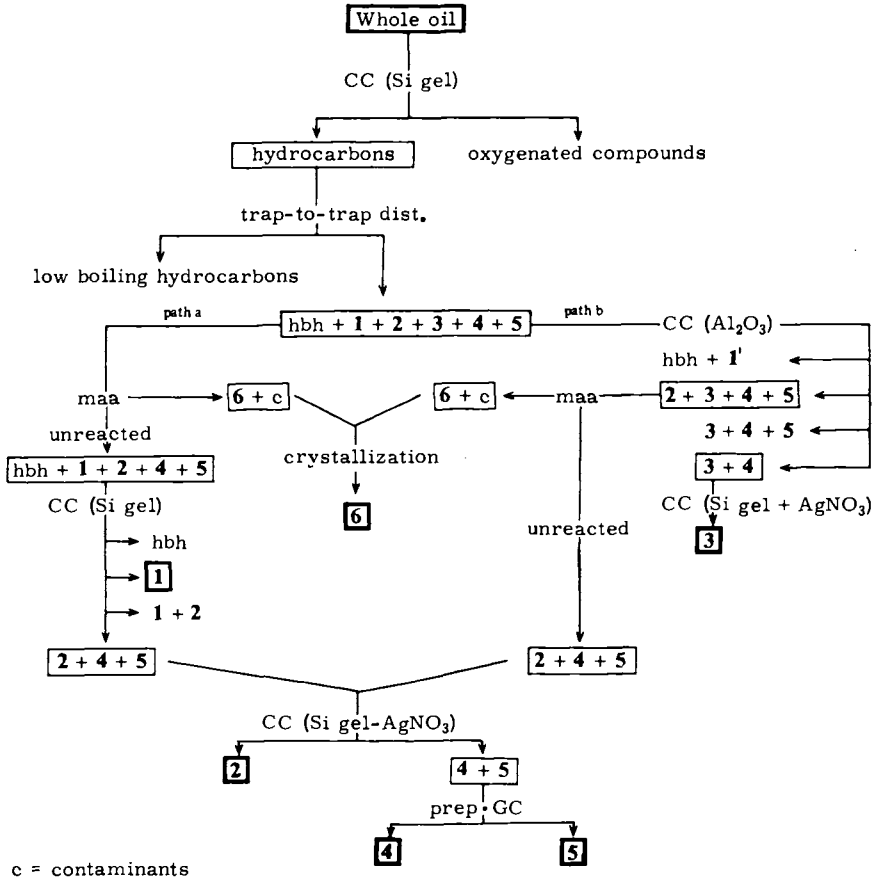
The same necessity arose for the structure elucidation of **2** and **3**, where a geometrical isomerism has been reasonably hypothized as making the difference.

To meet these requirements the high-boiling hydrocarbon fraction of *Oenanthe aquatica* fruit essential oil was newly investigated, optimizing an isolation procedure for polyynes, according to the sequence described in scheme 1. Each separation stage was monitored by gas chromatography since the compounds involved proved to be thermally stable.

Solvent extraction of the fruit was preferred to steam distillation used in previous work for the sake of the heat-sensitive polyynes. The entire hydrocarbon fraction, obtained by silica gel column chromatography of the crude extract, was submitted to a molecular distillation procedure[5] to give a coarse separation of a low-boiling fraction

consisting almost exclusively of monoterpenes and a high-boiling fraction in which a very satisfactory enrichement of the polyynes was realized. The contaminants of the high-boiling fraction were sesquiterpenes and dillapiole.

Scheme 1



c = contaminants
maa = maleic anhydride adduction
hbh = high boiling hydrocarbons

1, **2**, and **3** were best isolated by two independent routes, one optimized to obtain **1** and **2** (scheme 1, path a) and the other aiming at **3** alone (scheme 1, path b). In path a, the Diels-Alder reaction with maleic anhydride was performed on the entire high-boiling fraction allowing complete removal of **3** and some other reacting impurities. Subsequent column chromatography of the unreacted compounds on silica gel and on silica gel impregnated with $AgNO_3$, gave pure **1** and **2**.

The fact that **3** reacted quantitatively with maleic anhydride gave a first indication of the *all-trans* structure of the diene system of its chromophore.

In path b, the use of alumina column chromatography was successful in obtaining **3**, with **4** as the sole impurity. Subsequent $AgNO_3$-silica gel column chromatography gave

pure **3**. It should be noted that alumina promoted the conversion of **1** to the isomer having an ene-diyn-ene chromophore (**1'**)[6] as observed by UV and MS measurements of a gaschromatographically isolated sample. Moreover, another fraction from the alumina column chromatography, containing **2**, **3**, **4**, and **5**, after removal of **3** by maleic anhydride, was utilized to obtain, *via* $AgNO_3$-silica gel column chromatography, **2** and a mixture of **4** and **5**. Collection of small amounts of pure **4** and **5**, later performed by preparative gas chromatography, allowed the recording of the UV and MS spectra of these minor components.

As is known, polyacetylenes are extremely sensitive to air, heat and light, especially in the condensed phase, so that the isolated compounds were well-preserved in dilute solution of the chromatographic eluents. Removal of the solvents and manipulation of the spectroscopic samples was performed by the procedure described in the experimental part. The high purity of the specimens obtained allowed us to record, together with good quality NMR spectra, IR spectra in crystalline state at liquid nitrogen temperature. This allowed the observation of the out-of-plane vibration band of the *cis*-double bond which, in the liquid phase, forms one broad band with the $-CH_2-$ rocking vibration.

The NMR spectrum of **1** (Table 1) was in agreement with the methylene-interrupted structure, except for the assignment of the *cis* or *trans* structure of the isolated double bond, due to the complete overlapping of the H_c, H_e, and H_f signals, which, in our experimental conditions (90 MHz), prevented us from ascertaining the $J_{e,f}$.

Table 1. NMR data. 90 MHz in $CDCl_3$; δ scale; coupling constants in Hz

Comp.	H_a	H_b	H_c	H_d	H_e	H_f	H_g
1	dd 1.82	dq 6.25	dm 5.55	d 3.02	m 5.3–5.7	m 5.3–5.7	m 2.1
2[a]	dd 1.82	dq 6.35	dm 5.57	dm 5.36	m 6.4–6.8	m 6.4–6.8	m 5.95
6	dd 1.82	dq 6.37	dq 5.55				

[a] H_h m 2.14.
1,2,6: $J_{a,b}$ = 7.0, $J_{a,c}$ = 1.6, $J_{b,c}$ = 16.0. **1**: $J_{d,e}$ = 5.0. **2**: $J_{d,e}$ = 11.0, $J_{f,g}$ = 15.0.

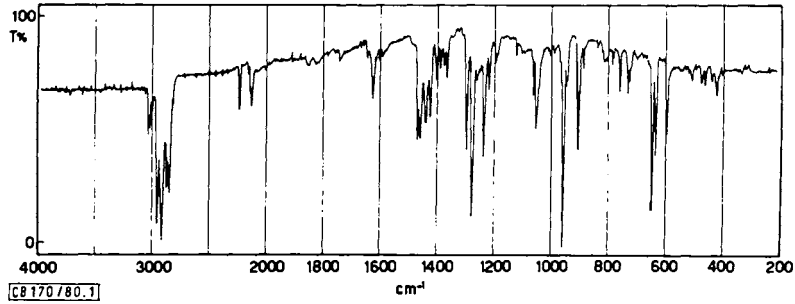


Fig. 1. IR spectrum of **1** (crystalline state)

The required information was obtained from the IR spectrum of the crystalline compound (Fig. 1). The band at 760 cm^{-1} together with the one at 1403 cm^{-1} were

respectively assigned to the out-of-plane and in-plane C–H deformations of a *cis*-double bond. It should be noted that the $-CH_2-$ rocking vibration splits into two components (728 and 732 cm^{-1}), not interfering at all with the comparably intense 760 cm^{-1} band. The structure of 2-*trans*-9-*cis*-2,9-pentadecadiene-4,6-diyne for **1** was then unambiguously verified.

$$\overset{a}{H_3}C-\overset{b}{C}H=\overset{c}{C}H-[C\equiv C]_2-\overset{d}{C}H_2-\overset{e}{C}H=\overset{f}{C}H-\overset{g}{C}H_2-[CH_2]_3-CH_3$$

1: *trans* *cis*

$$\overset{a}{H_3}C-\overset{b}{C}H=\overset{c}{C}H-[C\equiv C]_2-\overset{d}{C}H=\overset{e}{C}H-\overset{f}{C}H=\overset{g}{C}H-\overset{h}{C}H_2-[CH_2]_2-CH_3$$

2: *trans* *cis* *trans*

3: *trans* *trans* *trans*

4: *trans* *cis* or *trans* *cis*

5: *trans* *trans* or *cis* *cis*

6: Maleic anhydride adduct of **3**

As previously mentioned, UV spectra of both **2** and **3** evidenced the same chromophore (– = – ≡ – ≡ – = – = –). A comparison of IR spectra of **2** and **3** in the crystalline state (Fig. 2) was illuminating as regards the three double bonds of each chromophore. An exceptionally strong band at 750, together with a weaker one at 1407 cm^{-1}, clearly demonstrated a *cis*-double bond in the chromophore of **2**. These bands were completely lacking in the spectrum of **3**, while the $-CH_2-$ rocking band, split into two components (732 and 729 cm^{-1}), was again evident in both spectra, not interfering with the 750 cm^{-1} zone. On the other hand, the relative intensities of the two out-of-plane bending bands at 947 and 987 cm^{-1}, concerned, respectively, with

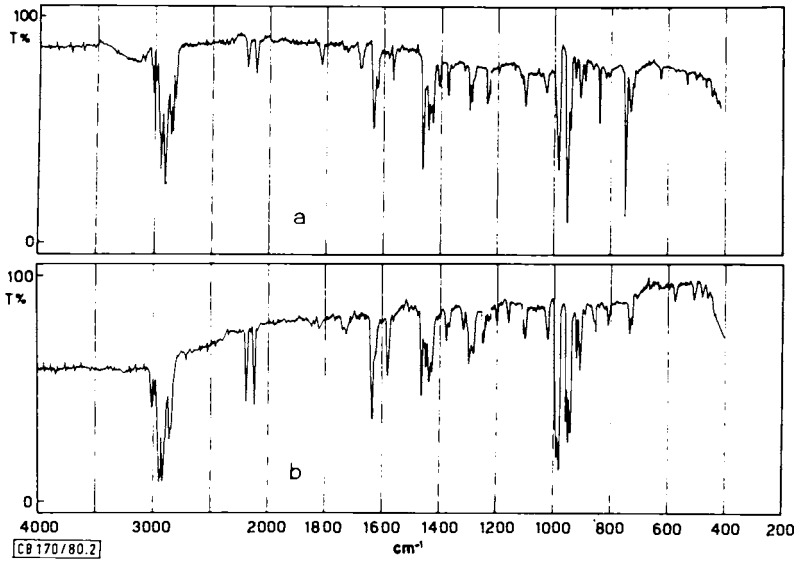


Fig. 2. IR spectra of **2** (a) and **3** (b) in the crystalline state

trans-bonds and with *trans*-bonds in olefinic conjugation, were markedly different in the two spectra. In particular, the 987 cm^{-1} band was more intense than 945 cm^{-1} band in the spectrum of **3**, while the contrary occured in the spectrum of **2**.

These data confirmed the *all-trans* structure of **3** and indicated that the *cis*-double bond of **2** was one of the two bonds of the conjugated diene system.

The NMR of **2** (Table 1) provided evidence for the chromophore position, showing the methyl-terminated $CH_3-CH=CH-C\equiv$ grouping. Moreover, additional evidence for the *cis*-double bond was obtained from NMR data, which also indicated its position in the chromophore. A doublet at $\delta = 5.36$, $J = 11$ Hz, in the extreme upfield side of the envelop of the olefinic hydrogen signals, was well observable and promptly assigned to the H_d atom.

A few more comments might be added concerning the NMR spectrum of **2**. Only the measurement of the $J_{e\text{-}f}$ coupling constant was hindered by the complete overlapping of the H_e and H_f signals. The dq pattern of H_b was well visible, even if partially submerged by the H_e, H_f envelop. The complex multiplet constituting the H_g signal lies in a zone ($\delta = 5.8-6.1$) free of overlapping and collapsed visibly in a broad doublet ($J = 15$ Hz) by the irradiation of the H_h-proton signal. The doublets arising from H_c and H_d, laying well-apart more upfield, gave no identification problem. Their assignments were well confirmed by irradiation procedures.

The collected data allowed us to conclusively recognize **2** as 2-*trans*-8-*cis*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne.

As stated previously, the *all-trans* configuration of the chromophore of **3** was evidenced by IR data and confirmed by the maleic anhydride adduction. The adduct itself (**6**), which crystallized in good yield and purity as a by-product of the isolation procedure (Scheme 1), was used to verify the chromophore position in the C_{15} chain of **3**. Indeed, neat signals for $CH_3-CH=CH-C\equiv$ grouping (Table 1) were exhibited by the quite simple NMR spectrum of **6**. The structure of *all-trans*-2,8,10-pentadecatriene-4,6-diyne was then unambiguously assigned to **3**. This already known hydrocarbon had been detected in the roots of *Oenanthe crocata*[3] and successively in the roots of *Oenanthe aquatica* itself[7].

Unfortunately, we were not able to record NMR and IR spectra of **4** and **5**, due to their presence at trace levels. However, UV and mass spectra revealed **4** and **5** as C_{15} polyynes with the same ene-diyne-diene chromophore. Consequently, these hydrocarbons were reasonably recognized as the remaining two 2-*trans*-8,10-pentadecatriene-4,6-diyne stereoisomers (i. e. 8-*cis*,10-*cis* and 8-*trans*,10-*cis*,alternatively).

The structure of the above-described hydrocarbons supported the generally accepted C_{15} biogenetic route parallelling that of C_{17} compounds[2b]. In particular **1**, already simply hypothized as a crucial point in the biosynthetic pathway, has been isolated and fully characterized. The subsequent oxidation and dehydration stages leading to 2-*trans*-8,10-pentadecatriene-4,6-diyne have been thoroughly demonstrated by the existence of all possible geometric isomers. In fact, the involved allylic oxidation accompanied by allylic rearrangement, which by successive dehydration leads to the ene-diyne-diene structure, appeared to be non-stereospecific, giving **2**, **4**, and **5**, in addition to the already known **3**.

In our opinion, further work on the oxygenated compounds connected with the above mentioned biogenetic route, deserves to be carried out.

The support of the grant Cap. 11/01, *Consiglio di Amministrazione dell'Università degli Studi di Firenze*, is gratefully acknowledged. The authors would like to thank Dr. *G. Moneti* for mass spectral measurements and Dr. *R. Hoffmann* for technical assistance. Thanks are also due to *Istituto Farmaceutico Militare, Firenze*, for making the extraction equipment available.

Experimental Part

Melting points are uncorrected. – Gas chromatography: Perkin-Elmer F 30 (FID), 1.9 m × 2 mm ID silanized glass columns, 5% SP 1000 on 100/120 Supelcoport (Supelco cat. 1 – 1797), 2.2 m × 2 mm ID inox columns, 12% DEGS on 80/90 Anakrom ABS, carrier N_2. – Gas chromatographic micropreparative work: Perkin-Elmer F 21 (FID) gas chromatograph, 1.8 m × 1/4'' glass column, 5% Carbowax 20 M on 90/100 Anakrom ABS, carrier N_2. – Mass spectra: Perkin-Elmer 270/B GC-MS instrument, 70 eV, 150 °C room temperature, utilizing the same columns as for F 30 gas chromatograph. – UV spectra: Perkin-Elmer 124 spectrophotometer. – ^1H NMR: Perkin-Elmer R 32 spectrometer, in $CDCl_3$, TMS as internal standard. – IR spectra: Perkin-Elmer 225 spectrophotometer.

Extraction and isolation: Ground *Oenanthe aquatica* fruit (5 kg) was extracted by percolation with light petroleum (40 – 60 °C) (35 l) and the volume was reduced (1 l) by removing the major part of the solvent in a rotary evaporator at 30 – 35 °C. An aliquot (100 ml) was submitted to column chromatography on 1 kg of silica gel 60 (Merck, 70 – 230 mesh) using light petroleum (40 – 60 °C)/diethyl ether (95 : 5) to elute the hydrocarbons. The concentrated eluate was submitted to a trap-to-trap distillation at 10^{-2} torr (evaporator at room temperature and condenser at – 190 °C) to separate the high-boiling from the low-boiling hydrocarbons[5]. The isolated high-boiling hydrocarbons were divided in two aliquots. One of these (scheme 1, path a) was treated with an excess of maleic anhydride in the usual manner. The adduct of **3** was removed and the unreacted residue (ca. 2 g) was separated by a 3 × 30 cm silica gel 60 (Merck, 230 – 400 mesh) column and cyclohexane as eluent, giving pure **1** (ca. 20 mg) and a **2** + **4** + **5** mixture.

In scheme 1, path b, the second aliquot of the high-boiling hydrocarbons was roughly fractionated on a 4 × 50 cm neutral alumina (Fluka 507 C) column eluting with cyclohexane. Five fractions were collected. The last fraction eluted, containing **3** in a substantial amount, was further chromatographed on a 2.5 × 35 cm, $AgNO_3$ impregnated (25%) and deactivated (6% H_2O), silica gel 60 (Merck, 230 – 400 mesh) column to give pure **3** (ca. 30 mg). An intermediate fraction containing **2** + **3** + **4** + **5** was worked-up *via* maleic anhydride adduction. The unreacted material, consisting of **2** + **4** + **5**, was combined with the corresponding fraction from path a. Further chromatographic fractionation of **2** + **4** + **5** mixture was performed by a 2.5 × 30 cm column packed with $AgNO_3$ impregnated (25%) and deactivated (6% H_2O) silica gel 60 (Merck, 230 – 400 mesh) using cyclohexane as eluent. This gave ca. 25 mg of pure **2** and a **4** + **5** mixture. Solutions of pure **4** and **5** for UV measurement were obtained by direct bubbling in cyclohexane of the corresponding gas chromatographic eluates in a micropreparative run carried out at 200 °C on the F 21 instrument.

NMR and IR sample preparation: The following procedure was used for **1**, **2**, and **3**. Each substance, in dilute solution of chromatographic solvent, was introduced in the evaporation pot (A) of the device illustrated in fig. 3. After assembling with B, the complete removal of solvent was obtained at 10^{-2} torr, maintaining the evaporator at 30 °C and the condenser at – 190 °C.

Atmospheric pressure was then restored with nitrogen and NMR sample solutions were prepared by direct introduction of $CDCl_3$ in the evaporation pot.

After the recording of NMR spectra, the $CDCl_3$ solutions were recovered and re-introduced in the evaporation pot to eliminate $CDCl_3$, in the A + B configuration. Atmospheric pressure was again restored with nitrogen and the evaporation pot quickly connected to the ball joint fitted on the side of a conventional low temperature infrared cell (A + C configuration). The deposition of the sample on the infrared cell window was obtained under vacuum by a trap-to-trap distillation process controlled by liquid nitrogen in the cell and thermostated water in the evaporation pot jacket. The samples, at first in vitreous state, were then crystallized by careful annealing. Following the infrared measurements, the polyynes were immediately recovered from the cell window and checked for purity by gas chromatography to confirm the dependability of the entire procedure.

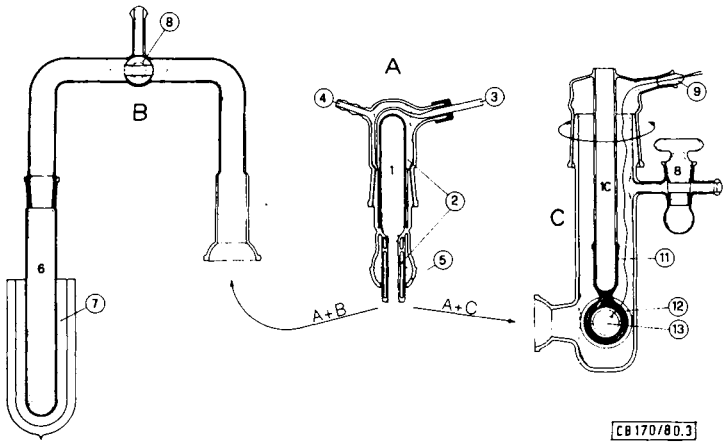


Fig. 3. Glass apparatus for spectroscopic sample preparation.
1 evaporation pot, 2 water jacket, 3 water inlet, 4 water outlet, 5 50/30 spherical joint, 6 condenser, 7 Dewar flask, 8 high vacuum stopcocks, 9 thermocouple, 10 cold finger, 11 glass-metal seal, 12 CsI external window, 13 CsI cold window

Lipid extraction and fatty acid composition determination: The *Folch* method[8] was employed for lipid extraction. The lipid classes were separated by TLC (Merck silica gel 60) according to *Malins* and *Mangold*[9]. The triglyceride fraction was analyzed for fatty acid composition by gas chromatography of the methyl esters using 12% DEGS column at 180 °C.

2-trans-9-cis-2,9-Pentadecadiene-4,6-diyne (**1**): Colorless oil. – UV: λ_{max} (methanol) = 282, 267, 253, 240 nm (ene-diyne). – IR (crystal): C≡C 2235, 2129, CH=CH 1625, *trans* conj. CH=CH 958, *cis* CH=CH 1403, 760, CH_2 rock 732, 728, crystalline state 647, 635, 595. – MS: M^+ *m/z* = 200 (6%), 157 (15), 143 (56), 128 (100), 115 (61), 91 (35), 77 (37), 63 (21), 41 (35).

2-trans-8-cis-10-trans-2,8,10-Pentadecatriene-4,6-diyne (**2**): Colorless oil. – UV: λ_{max} (methanol) = 334, 314, 296, 277, 266, 250 nm (ene-diyne-diene). – IR (crystal): C≡C 2195, 2123, CH=CH 1625, $[CH=CH]_2$ 1634, 1570, *trans* CH=CH 947, *trans* CH=CH (olefinic conjugation) 983, *cis* CH=CH 1408, 750, CH_2 rock 732, 729. – MS: M^+ *m/z* = 198 (13%), 183 (17), 169 (13), 155 (42), 153 (45), 141 (100), 128 (45), 115 (71), 91 (19), 77 (26).

2-trans-8-trans-10-trans-2,8,10-Pentadecatriene-4,6-diyne (**3**): Colorless oil. – UV: λ_{max} (methanol) = 337, 315, 296, 266, 250 nm (ene-diyne-diene). – IR (crystal): C≡C 2198, 2123,

CH = CH 1624 (sh), $[CH=CH]_2$ 1634, 1582, *trans* CH = CH 945, *all-trans* $[CH=CH]_2$ 991, 984, CH_2 rock 728, 733. – MS: M^+ *m/z* = 198 (92%), 183 (4), 155 (38), 153 (47), 141 (100), 128 (55), 115 (84), 91 (21), 77 (34).

Maleic anhydride adduct of **3** (**6**): Maleic anhydride adduction was performed as indicated in scheme 1, according to *Anet*[10]. The separated adduct was recrystallized from ethyl ether as colorless needles, m. p. 113 °C. – UV: λ_{max} (methanol) = 282, 267, 253, 240 nm (ene-diyne). – IR (nujol): C ≡ C 2232, 2148, C = O 1850, 1780, CH = CH 1635, *trans* CH = CH 945. – MS: M^+ *m/z* = 296 (40%), 281 (10), 267 (12), 253 (9), 239 (15), 225 (20), 211 (40), 198 (65), 181 (25), 165 (70), 155 (60), 141 (100), 128 (55), 115 (90), 91 (70), 77 (85).

$C_{19}H_{20}O_3$ (296.3) Calcd. C 77.02 H 6.75 Found C 77.00 H 6.70

2,8,10-Pentadecatriene-4,6-diyne (**4**): – UV: λ_{max} (cyclohexane) = 338, 316, 297, 267, 252 nm (ene-diyne-diene). – MS: M^+ *m/z* = 198 (17%), 183 (11), 169 (13), 155 (41), 153 (48) 141 (100), 128 (52), 115 (79), 91 (23), 77 (35).

2,8,10-Pentadecatriene-4,6-diyne (**5**): – UV: λ_{max} (cyclohexane) = 340, 317, 297, 267, 257 nm (ene-diyne-diene). – MS: M^+ *m/z* = 198 (89%), 155 (30), 153 (50), 141 (100), 128 (54), 115 (83), 91 (23), 77 (36).

[1] *F. F. Vincieri, S. A. Coran,* and *M. Bambagiotti A.*, Planta Med. **29**, 101 (1976).
[2] *F. Bohlmann, T. Burkhardt,* and *C. Zdero,* Naturally Occurring Acetylenes, Academic Press, London 1973. – [2a] p. 27. – [2b] p. 222. – [2c] p. 230.
[3] *F. Bohlmann* and *K. Rode,* Chem. Ber. **101**, 1163 (1968).
[4] *F. Bohlmann* and *K. Rode,* Chem. Ber. **101**, 1889 (1968).
[5] *F. F. Vincieri, M. Bambagiotti A.,* and *S. A. Coran,* Chim. Ind. (Milan) **54**, 824 (1972).
[6] *W. Oroshnik, A. D. Mebane,* and *G. Karmas,* J. Am. Chem. Soc. **75**, 1050 (1953).
[7] *F. Bohlmann, C. Zdero, J. Trénel, P. Hanel,* and *M. Grenz,* Chem. Ber. **104**, 1322 (1971).
[8] *J. Folch, M. Lees,* and *G. M. Sloane-Stanley,* J. Biol. Chem. **226**, 497 (1957).
[9] *D. C. Malins* and *H. K. Mangold,* J. Am. Oil Chem. Soc. **37**, 576 (1960).
[10] *E. F. L. J. Anet, B. Lythgoe, M. H. Silk,* and *S. Trippett,* J. Chem. Soc. **1953**, 309.

[170/80]