Natural Acetylenes. Part XLII.[1] Novel C_7, C_8, C_9, and C_{10} Polyacetylenes From Fungal Cultures [2]

By **Milton T. W. Hearn, Sir Ewart R. H. Jones,* Martin G. Pellatt, Viktor Thaller,** and **John L. Turner,**
The Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY

The diynol (−)-EtCH(OH)·[C≡C]$_2$H has been found in *Gymnopilus spectabilis*, the triynol and triynoic acid Me[C≡C]$_3$R (R = CH$_2$·OH or CO$_2$H) have been found in *Psilocybe merdaria*, *Kuehneromyces mutabilis*, *Russula vesca*, and *Ramaria flava*, and the acids H[C≡C]$_3$·CH$_2$·CH$_2$·CO$_2$H and HO$_2$C·CH=CH·[C≡C]$_2$·CH$_2$·CH$_2$·CO$_2$H have been obtained from *Serpula lacrymans* cultures. The Strophariaceae, Russulaceae, and Gomphaceae families have not been reported previously as polyacetylene producers.

As part of a continuing study of the polyacetylene content of Basidiomycete fungi we have examined the culture fluids of species belonging to families not previously or only sporadically † reported as polyacetylene producers. The first natural C_7 polyacetylene and two C_8 polyacetylenes, both not previously obtained from natural sources, have now been isolated. In addition, a C_9 and a C_{10} polyacetylenic acid were detected as trace components of a well-investigated fungal culture.

Gymnopilus spectabilis (Fr.) Singer, Cortinariaceae family † contained polyacetylenes only in the neutral

† *Flammula sapinea* (Fr.) Kummer, already known to be a producer of allenic polyacetylenes, has now also been assigned to this family and is called *Gymnopilus sapineus* (Fr.) Maire (*cf.* R. Singer, 'The Agaricales in Modern Taxonomy,' J. Cramer, Weinheim, 1962).

[1] Part XLI, I. W. Farrell, J. W. Keeping, M. G. Pellatt, and V. Thaller, *J.C.S. Perkin I*, 1973, 2642.

[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.

fraction with (—)-hepta-1,3-diyn-5-ol (I) as the main constituent (57 mg l^{-1} culture fluid). Three more polar-enediynes were obtained in trace amounts and were not identified. The diynol (I) was also found to be the main, though less abundant, polyacetylene of *G. hybridus*. The natural product and the synthetic (±)-alcohol (I) [3] had identical spectral and chromatographic properties.

The triynol (II) and the triyne acid (III; R = H), isolated as the methyl ester (R = Me), were found to be the main polyacetylenes of *Psilocybe merdaria* (Fr.) Ricken * (4·5 and 3·7 mg l^{-1} culture fluid), *Kuehneromyces mutabilis* (Schaeffer, Fries) Sing. and Sm. (2 and 1·5 mg l^{-1} culture fluid) (both Strophariaceae family), *Russula vesca* Fr. (3·5 and 2 mg l^{-1} culture fluid) (Russulaceae family), and *Ramaria flava* (Schaeffer non Fr.) Quel. (5 and 0·9 mg l^{-1} culture fluid) (Gomphaceae family). The natural products and the synthetic alcohol (II) [4] and ester (III; R = Me) [5,6] were identical. Small amounts of other polyacetylenes were detected in all extracts, some with terminal acetylene groups [the C_7 triyne alcohol (IV) appeared to be a likely minor component on the basis of its u.v. and i.r. spectra and chromatographic behaviour] but they were not identified.

The isolation of the alcohol (II) and acid (III; R = H) brings the number of known natural C_8 polyacetylenes up to eight. The two new natural products appear to arise from straightforward chain shortening of the most unsaturated C_{18} intermediate (V) in Bu'Lock's biogenetic

$$EtCH(OH)\cdot[C{\equiv}C]_2H \quad (I)$$

$$Me[C{\equiv}C]_3\cdot CH_2OH \quad (II)$$

$$Me[C{\equiv}C]_3\cdot CO_2R \quad (III)$$

$$H[C{\equiv}C]_3\cdot CH_2OH \quad (IV)$$

$$Me[C{\equiv}C]_3\cdot CH_2\cdot CH^{c}{=}CH\cdot[CH_2]_7\cdot CO_2R \quad (V)$$

$$RO_2C\cdot[C{\equiv}C]_3\cdot CH_2\cdot CH_2\cdot CO_2R \quad (VI)$$

$$RO_2C\cdot CH^{t}{=}CH\cdot[C{\equiv}C]_2\cdot CH_2\cdot CH_2\cdot CO_2R \quad (VII)$$

$$H[C{\equiv}C]_3\cdot CH_2\cdot CH_2\cdot CO_2R \quad (VIII)$$

$$RO_2C\cdot CH^{c}{=}CH\cdot[C{\equiv}C]_2\cdot CH_2\cdot CH_2\cdot CO_2R \quad (IX)$$

scheme,[7] leaving the distal C_8 chain intact. Although not detected till now, such metabolites might be expected to be widely distributed amongst polyacetylene-producing fungi: their occurrence in the widely differing Strophariaceae, Russulaceae, and Gomphaceae families, not reported previously as polyacetylene producers, favours this view. On the other hand, there might be some taxonomical significance in the appearance of the alcohol (II) and the acid (III; R = H) in the two subfamilies (Stropharioideae-*P. merdaria* and Philiotoideae-*K. mutabilis*) of the Strophariaceae: further species will have to be screened to explore this possibility.

Serpula lacrymans Pers. ex S. F. Gray † was found [8] to contain the diacids (VI; R = H) and (VII; R = H). This fungus has been reinvestigated in connection with biosynthetic experiments, and traces of two new natural polyacetylenes, the C_9 acid (VIII; R = H) and the C_{10} diacid (IX; R = H) have now been isolated as the

$$(VI;\ R = H) \xrightarrow{i,\ ii} (VIII;\ R = Me)$$

Reagents: i, $(NH_3)_4CuSO_4$–Me_2CO; ii, MeOH–H_2SO_4

$$HO\cdot CH_2\cdot C{\equiv}CBr + HC{\equiv}C\cdot CH_2\cdot CH_2\cdot CO_2H$$

$$\downarrow i,\ ii$$

$$HO\cdot CH_2\cdot[C{\equiv}C]_2\cdot CH_2\cdot CH_2\cdot CO_2Me \quad (X)$$

$$\downarrow iii$$

$$OCH\cdot[C{\equiv}C]_2\cdot CH_2\cdot CH_2\cdot CO_2Me \quad (XI)$$

$$\downarrow iv$$

$$(VII) + (IX)$$

Reagents: i, CuCl–$NH_2\cdot OH$–$EtNH_2$; ii, MeOH–H_2SO_4; iii, MnO_2–CH_2Cl_2; iv, $MeO_2C\cdot\bar{C}H\cdot\overset{+}{P}Ph_3$

corresponding methyl esters (R = Me). Their structures were established by direct comparison with authentic specimens, which were synthesised by the routes indicated.

Enzymic decarboxylation of αβ-acetylenic acids with *Coprinus quadrifidus* cultures [9] was demonstrated with the di-acid (VI; R = H) when it was converted into the acid (VIII), which metabolite, however, is not produced by that fungus.

EXPERIMENTAL

Instruments used: u.v., Unicam SP 800 (spectra recorded in Et_2O unless stated otherwise); i.r., Unicam SP 1000 and Perkin-Elmer 257; n.m.r., Perkin-Elmer R10 and R14; mass spectra (direct insertion) Varian-MAT CH7; m.p.s (corr.), Kofler hot-stage apparatus.

Liquid chromatography: SiO_2 H.B.L. M60 in columns and Merck $HF_{254+366}$ in 0·3 mm (t.l.c.) and 1 mm (p.l.c.) layers, respectively.

Petrol refers to light petroleum of b.p. 30—40° (*G. spectabilis* work-up) or 40—60° (remaining work).

Growth and Extraction of Fungal Cultures and General

* *Psilocybe sarcocephala* is, according to Singer's classification, *Psatyrella sarcocephala* and belongs to the Coprinaceae family. Thus, no polyacetylenes have been reported in the genus Psilocybe, *sensu* Singer.

† This fungus has previously been classified as *Merulius lacrymans* Wulfen *ex* Fr.

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Work-up of Extracts.—The fungi were grown on 3% malt extract (for *G. spectabilis* see special medium). When maximum polyacetylene concentrations (estimated by u.v.) were reached, the media were decanted and the culture fluids were continuously extracted with Et_2O (48 h). The extracts were concentrated to 200—300 ml and separated into neutral and acid fractions ($NaHCO_3$), and the latter were esterified with 4% H_2SO_4 in MeOH.

Isolation of Polyacetylenes from Cultures of Gymnopilus spectabilis (*Fr.*) *Singer.*—The fungus was grown in shaken culture on a medium of glucose (4%), peptone (mycological Oxoid, 0·1%), KH_2PO_4 (0·1%), KCl (0·05%), $MgSO_4,7H_2O$ (0·05%), $FeSO_4$ (0·001%), and yeast extract (Oxoid, 0·2%) for 6 days in 12 flasks (7·2 l culture fluid). Only the neutral fraction showed polyacetylene absorption (u.v. and i.r.). The concentrated neutral fraction (540 mg) was separated on a SiO_2 column (60 g) by stepwise elution [petrol–Et_2O (2 : 1) to Et_2O–MeOH (19 : 1); 1·5 l] and 100 ml fractions were collected. Fractions 2 and 3 yielded the liquid (−)-hepta-1,3-diyn-5-ol (I) (410 mg), b.p. 45° (bath) at 0·2 mmHg (Found: C, 76·5; H, 7·25. C_7H_8O requires C, 77·75; H, 7·5%), $[\alpha]^{20}$ −14·4° (589 nm), −14·8° (578), −16·5° (546), −27·5° (436), and −40° (365) (*c* 0·575 in EtOH), $[\alpha]^{20}$ −24·4° (589 nm), −25·2° (578), −28·5° (546), −48° (436), and −73° (365) (*c* 1·035 in Et_2O), $\lambda_{max.}$ (EtOH) 252 (ε 440), 238 (675), 225·5 (750), and 217 (590) nm, $\nu_{max.}$ (CCl_4) 3660 (OH), 3340 (≡CH), 2250 and 2100 (C≡C), and 1050 (C–O) cm^{-1}, τ (CCl_4) 8·98 (t, *J* 7·5 Hz, $CH_3{\cdot}CH_2$), 8·27 (dq, *J* 7 and 7·5 Hz, $CH_3{\cdot}CH_2{\cdot}CHOH$), 7·93 (s, C≡CH), 7·38 (s, CHO*H*; disappears on addition of D_2O), and 5·71 (t, *J* 7 Hz, $CH_2{\cdot}CHOH$); the alcohol (I) was identical in all respects but rotation with (±)-hepta-1,3-diyn-5-ol [3] prepared from propanal and 1,4-dichlorobut-2-yne.

Fractions 5—13 were concentrated and the residue (21 mg) was separated by t.l.c. (Et_2O; 3 elutions) into several bands. The three central bands contained polar polyacetylenes (less than 1 mg each) with $\lambda_{max.}$ 284, 268, 253·5, 240, and 227infl. nm, $\lambda_{max.}$ 280, 268, 254, 241, and 227 infl. nm, and $\lambda_{max.}$ 281, 267, 254, 239, and 226 nm (quoted in order of increasing polarity).

The alcohol (I) was also found in *G. hybridus* (Fr. ex Fr.) Singer, was not detected in *G. penetrans* and *G. sapineus* extracts.

Isolation of Polyacetylenes from Cultures of Psilocybe merdaria (*Fr.*) *Ricken.*—The fungus was grown in static culture for 35 days in 30 flasks (23 l culture fluid).

Neutral fraction. The concentrated fraction (560 mg) was separated by p.l.c. (petrol–Et_2O, 3 : 1; 2 elutions) into 6 bands (A—F in order of increasing polarity). The least polar band A showed u.v. end absorption ($\lambda_{max.}$ 211 nm) and possibly contained small amounts (*ca.* 2 mg) of a triyne. The extracts from bands B—D contained the same major component (t.l.c.) and were combined, concentrated (192 mg), and further separated by p.l.c. (petrol–Et_2O, 1 : 1; 2 elutions) into three bands of which only the middle one contained acetylenic material (102 mg); this was crystallised (CCl_4) and gave plates of octa-2,4,6-triyn-1-ol (II) (85 mg), m.p. and mixed m.p. 92—94° (lit.,[4] 93°) which rapidly turned red in light, $\lambda_{max.}$ (EtOH) 310 (ε 131), 307 (122), 290·5 (207), 287 (188), 273·5 (188), 269 (183), 259 (141), 254 (146), 240 (94), and 209 (127,000) nm, τ (CCl_4) 8·13br (1H, OH), 8·0 (3H, s, $CH_3{\cdot}C{\equiv}C$), and 5·72 (2H, s, $C{\equiv}C{\cdot}CH_2{\cdot}OH$), *m/e* 118 ($M^+$, 100%), 101 (50), 98 (20), 90 (49), 89 (71), 75 (56), and 63 (64). A sample of the alcohol (II) was prepared from penta-1,3-diyne and 3-bromoprop-2-yn-1-ol by Chodkiewicz coupling under standard conditions (see below) in 60% yield.[4] Band E possibly also contained a triyne ($\lambda_{max.}$ 211 nm; *ca.* 1 mg) which was not isolated. Band F contained *ca.* 6 mg of an enediyne, $\lambda_{max.}$ 282, 266, 252, 239, 227, and 213 nm together with non-acetylenic material (150 mg). Repeated p.l.c. in several solvent systems achieved little enrichment of the polyacetylene and precluded identification.

Acid fraction. The esters were chromatographed on a SiO_2 column (25 g) by stepwise elution [petrol–Et_2O (19 : 1) to Et_2O–MeOH (1 : 1); 1 l] and nine 100 ml fractions were collected. Only fractions 2 and 3 (eluted with 5—10% Et_2O in petrol) contained polyacetylenes: they were combined and separated by p.l.c. (petrol–Et_2O, 19 : 1; 3 elutions) into 2 major bands of which the less polar showed polyacetylene absorption and yielded on crystallisaton (petrol) blades of methyl octa-2,4,6-triynoate (III; R = Me) (84 mg), m.p. and mixed m.p. 56—58° (lit.,[5] 53—56°; lit.,[6] 57°) (Found: C, 74·0; H, 4·1. Calc. for $C_9H_6O_2$: C, 73·55; H, 4·26%), τ (CCl_4) 7·95 (3H, s, $CH_3{\cdot}C{\equiv}C$) and 6·23 (3H, s, $CO_2{\cdot}CH_3$), *m/e* 146 (M^+, 80%), 115 (100), 103 (11), 87 (39), and 61 (15). I.r. and u.v. spectra were identical with those of an authentic specimen prepared by esterification (MeOH–H_2SO_4) of the product of coupling between bromopropiolic acid and penta-1,3-diyne (see below for the conditions used in Chodkiewicz couplings).

Isolation of Polyacetylenes from Cultures of Kuehneromyces mutabilis (*Schaeffer, Fries*) *Sing. and Sm.*—The fungus was grown in static culture for 46 days in 30 flasks (22·5 l culture fluid).

Neutral fraction. The concentrated fraction (420 mg) was separated by column chromatography (150 g SiO_2) by stepwise elution (petrol to Et_2O). The single triyne-containing fraction was further separated by p.l.c. (petrol–Et_2O, 3 : 2; 2 elutions). The major band yielded on crystallisation octa-2,4,6-triyn-1-ol (II) (45 mg). A more polar minor band contained a triyne (*ca.* 1 mg), $\lambda_{max.}$ 210 nm, $\nu_{max.}$ (CCl_4) 3300 (≡C–H) and 2250 cm^{-1} (C≡C).

Acid fraction. The methyl esters (780 mg) were first separated on a column (as above) and the single acetylene-containing fraction was further purified by p.l.c. (petrol–Et_2O, 9 : 1; 4 elutions) yielding on crystallisation the triyne ester (III; R = Me) (34 mg). A more polar band yielded a small amount (less than 2 mg) of a triyne ester with the same u.v. absorption as the ester (III).

Isolation of Polyacetylenes from Cultures of Ramaria flava (*Schaeffer non Fr.*) *Quel.*—The fungus was grown in shaken culture for 14 days in 18 flasks (13·5 l culture fluid).

Neutral fraction. The concentrated fraction (240 mg) was separated by p.l.c. (petrol–Et_2O, 1 : 1) into four bands (A—D, in order of increasing polarity). The main polyacetylene-containing band (B) yielded a solid (102 mg) from which on crystallisation (CCl_4) octa-2,4,6-triyn-1-ol (II) (89 mg) was obtained [band A yielded an additional 3 mg of the alcohol (II) on p.l.c. (petrol–Et_2O, 1 : 1)]. The combined and concentrated mother liquor from the above crystallisation and the extract from band C (20 mg) were separated (p.l.c., petrol–Et_2O, 3 : 1) into three bands of which the least polar (4 mg) probably contained an enediyne (*ca.* 2 mg), $\lambda_{max.}$ 282, 267, 253, 240, and 210 nm, $\nu_{max.}$ (CCl_4) 3300 (≡C–H) and 2210 cm^{-1} (C≡C), and the most polar band (3 mg) probably a triyne (*ca.* 1 mg), $\lambda_{max.}$ 209 nm, $\nu_{max.}$ (CCl_4) 3300 (≡C–H) and 2210 cm^{-1} (C≡C). Band D (92 mg) contained *ca.* 1 mg of a triyne, $\lambda_{max.}$ 210 nm.

Acid fraction. The esters (50 mg) were separated by

p.l.c. (petrol–Et_2O, 9 : 1; 2 elutions) into three bands of which the least polar yielded methyl octa-2,4,6-triynoate (III; R = Me) (15 mg) and the middle one traces of a polyacetylene with $\lambda_{max.}$ 305, 282, and 270 nm; the most polar band contained no polyacetylenes.

Isolation of Polyacetylenes from Cultures of Russula vesca *Fr.*—The fungus was grown in static culture for 43 days in 30 flasks (22·5 l culture fluid).

Neutral fraction. The concentrated fraction (250 mg) was separated by p.l.c. (petrol–Et_2O, 3 : 2; 2 elutions) into seven bands (A—G, in order of increasing polarity). The major band (B) gave on further p.l.c. and crystallisation (CCl_4) octa-2,4,6-triyn-1-ol (II) (75 mg). The combined extracts from bands C—E were further separated (p.l.c., petrol–Et_2O, 3 : 2; 4 elutions) and yielded more of the alcohol (II) (31 mg) and a band which in addition to the alcohol (II) contained a slightly more polar triyne, $\lambda_{max.}$ 210 nm, which could not be separated.

Acid fraction. The methyl esters were separated by p.l.c. (petrol–Et_2O, 2 : 1; 2 elutions) into several bands. From the second least polar band methyl octa-2,4,6-triynoate (III; R = Me) (65 mg) was isolated.

Isolation of Polyacetylenes from Cultures of Serpula lacrymans *Pers. ex S. F. Gray.*—The fungus was grown in surface culture for 48 days in 5 flasks (4 l culture fluid). The methyl esters were chromatographed (p.l.c., petrol–Et_2O, 4 : 1) and the polyacetylene-containing band was further separated (p.l.c., petrol–Et_2O, 5 : 1; 3 elutions) into four closely running bands (A—D, in order of increasing polarity). Each band was further separated by repeated t.l.c. and p.l.c. Band A gave methyl nona-4,6,8-triynoate (VIII; R = Me) (*ca.* 0·2 mg), band B the *trans*-enediyne diester (VII; R = Me) [8] (*ca.* 1 mg), band C the triyne diester (VI; R = Me) [8] (63 mg), and band D the *cis*-enediyne diester (IX; R = Me) (*ca.* 0·5 mg). All metabolites were compared with authentic specimens, the minor constituents by R_F, u.v., and mass spectra. The C_9 ester (VIII; R = Me) and the *cis*-diester (IX; R = Me) were synthesised for this purpose.

Synthesis of Methyl Nona-4,6,8-triynoate (VIII; R = Me).—Tetra-ammine copper sulphate [10] (1 g) was added in portions over 5 min to the crude natural diacid (VI; R = H) (*ca.* 70 mg) in stirred, refluxing Me_2CO (50 ml). Stirring and heating was continued for 5 min; the mixture was cooled, filtered, and concentrated. The residue was esterified (MeOH–H_2SO_4) and the crude methyl esters were separated by p.l.c. (petrol–Et_2O, 4 : 1; 2 elutions). The band with R_F 0·5 gave the *triyne ester* (VIII; R = Me), plates (32 mg), m.p. 35—36° (from petrol), $\lambda_{max.}$ (EtOH) 306infl. (ε 163), 303 (183), 294 (137), 287infl. (236), 284 (280), 279infl. (173), 270infl. (207), 268 (250), 263infl. (180), 252·5 (173), 283·5 (140), and 205·5 (133,500) nm, $\nu_{max.}$ (CCl_4) 3310 (C≡CH), 2220 (C≡C), and 1750 (ester CO) cm^{-1}, τ (CCl_4) 8·04 (1H, s, C≡CH), 7·44 (4H, m, CH_2·CH_2·CO_2Me), and 6·34 (3H, s, CO_2·CH_3), *m/e* 160 (M^+, 80%), 149 (42), 143 (21), 129 (25), 107 (41), 101 (43), 91 (66), 89 (26), 87 (100), and 75 (44).

Synthesis of Dimethyl Dec-cis-2-*ene*-4,6-*diynedioate* (IX; R = Me).—To CuCl (20 mg), NH_2OH,HCl (700 ml), $EtNH_2$ (40% in H_2O, 8 ml), and MeOH (5 ml) stirred under N_2 at 20° was added first pent-4-ynoic acid (400 mg, 5 mmol) in MeOH (10 ml), and then, after 5 min, 3-bromoprop-2-yn-1-ol (675 mg, 5 mmol) in MeOH (10 ml) (dropwise). After further stirring (1·5 h), ice (150 g), water (100 ml), and KCN (1 g) were added to the mixture. Extraction with Et_2O and $NaHCO_3$ yielded the hydroxy-acid (600 mg), which was converted (MeOH–H_2SO_4) into the hydroxy-ester (X), b.p. 120—124° at 0·1 mmHg, n_D^{20} 1·5227 (lit.,[11] b.p. 115—120° at 0·08 mmHg, n_D^{20} 1·5223), $\nu_{max.}$ (CCl_4) 3630 (OH free), 3520 (OH bonded), 2280 (C≡C), and 1750 (ester CO) cm^{-1}, τ (CCl_4) 7·5 (4H, s, CH_2·CH_2·CO_2Me), 6·38 (3H, s, CO_2·CH_3), and 5·77 (s, 2H, CH_2OH). The signal at τ 7·5 was resolved into a multiplet on addition of benzene.

The hydroxy-ester (X) (100 mg, 0·6 mmol) and MnO_2 (1·0 g) in CH_2Cl_2 (25 ml) were shaken for 4 h and gave on work-up the aldehyde ester (XI), an unstable oil (91 mg, 0·55 mmol, 91%), $\lambda_{max.}$ 286 (rel. *E* 2·4), 270 (2·9), 255·5 (1·95), and 242·5 (1·0) nm. This was immediately dissolved in CH_2Cl_2 (1 ml) and added dropwise to methoxycarbonylmethylenetriphenylphosphorane [12] (182 mg, 0·55 mmol) stirred in CH_2Cl_2 (2 ml) at −15°. Stirring was continued, first for 0·5 h at −15°, and then for another h without cooling (warming up to 20°). The mixture was concentrated and the residue separated by p.l.c. (petrol–Et_2O, 1 : 1; 2 elutions). The band with R_F 0·6 gave on crystallisation (petrol) the *trans*-diester (VII; R = Me) (47 mg), m.p. and mixed m.p. 57—58° (lit.,[13] 56·6—58°), τ (CCl_4) 7·42 (4H, m, CH_2·CH_2·CO_2Me), 6·34 (3H, s, CH_2·CO_2·CH_3), 6·29 (3H, s, CH=CH·CO_2·CH_3), 3·78 (1H, d, *J* 15·9 Hz, *trans*-CH=CH·CO_2Me), and 3·32 (1H, dt, *J* 15·9 and 1 Hz, *trans*-*CH*=CH·CO_2Me), *m/e* 220 (M^+, 100%), 205 (15), 189 (41), 177 (54), 161 (80), 149 (30), 119 (24), and 87 (30). The band with R_F 0·5 gave on crystallisation (petrol) the cis-*diester* (IX; R = Me) (18 mg), m.p. 46—46·5° (Found: C, 65·6; H, 5·6. $C_{12}H_{12}O_4$ requires C, 65·4; H, 5·5%), $\lambda_{max.}$ (EtOH) 307 (ε 14·300), 288 (15,200), 274 (9450), 259infl. (4600), 224 (26,900), and 215 (22,200) nm, $\nu_{max.}$ (CCl_4) 2250 and 2160 (C≡C), 1750 (non-conj. ester CO), 1730 (conj. ester CO), and 1615 (CH=CH) cm^{-1}, $\nu_{max.}$ (CS_2) 820 (*cis*-CH=CH) cm^{-1}, τ (CCl_4) 7·4 (m, CH_2·CH_2·CO_2Me), 6·34 (s, CH_2·CO_2·CH_3), 6·28 (s, CH=CH·CO_2·CH_3), and 3·93 (s, *cis*-*CH*=*CH*·CO_2Me), *m/e* 220 (M^+, 47%), 205 (10), 189 (25), 177 (23), 161 (100), 147 (21), 119 (25), and 87 (20).

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