Phytochemistry, Vol. 27, No. 10, pp. 3169 3173, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00
Pergamon Press plc.

A NEW FATTY ACID METHYL ESTER AND OTHER BIOLOGICALLY ACTIVE COMPOUNDS FROM *ASPERGILLUS NIGER*

MURALEEDHARAN G. NAIR* and BASIL A. BURKE

Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568, U.S.A.

(*Received* 23 *December* 1987)

Key Word Index—*Aspergillus niger*; fungus; methyl 3-methyl-8-hydroxy-4-decenoate; phenylethanol; phenylacetic acid; phenoxyacetic acid; *p*-methoxyphenylacetic acid; mannitol; citric acid.

Abstract—Methyl 3-methyl-8-hydroxy-4-decenoate, a new C_{10} fatty acid methyl ester, was isolated and characterized from *Aspergillus niger* var. Tieghem. The chemical synthesis of this compound has also been achieved. Other compounds characterized from *A. niger* were phenylethanol, phenylacetic acid, phenoxyacetic acid, *p*-methoxyphenylacetic acid, mannitol and citric acid. All compounds except mannitol, inhibited the germination of cress and lettuce seeds. Antifungal bioassay of the above compounds on *Cladosporium herbarum* showed activity except for the C_{10} fatty acid methyl ester, mannitol and citric acid.

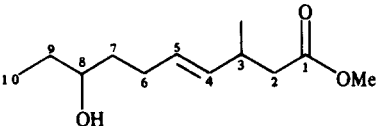
INTRODUCTION

During our study of the allelochemistry exhibited by *Kalanchoe daigremontiana* [1], some of the young plantlets were found to be infected by a fungus which led to their decay. This fungus was subsequently identified as *Aspergillus niger* var. Tieghem, and it was bioassayed using a velvetleaf (*Abutilon theophrasti*) seed germination assay to investigate the possible use of it or its metabolite as a means of controlling velvetleaf, a perennial weed which is a serious problem to cotton, corn and soybean. Our interest was to isolate possible herbicidal compounds from this *A. niger* species, which could control velvetleaf. *Aspergillus niger* sp. are well known for their citric acid production [2–4]. Biologically active orlandin, a plant growth inhibitor [5], other polyketide compounds [6–8], piperazine related compounds [9], peptides [10, 11] and other miscellaneous compounds [12, 13] are also reported from *A. niger*. Monoglucosyloxyoctadecanoic acid, a fatty acid glucoside [14], was another metabolite reported from *Aspergillus* species. In our attempt to isolate herbicidally active compounds from *A. niger* grown on potato dextrose (PD) medium, we describe the characterization and synthesis of a new fatty acid methyl ester **1** as well as compounds **2**–**5** and their biological activity in tests on seed germination and fungal growth.

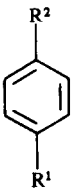
RESULTS AND DISCUSSION

The mycelium free PD broth in which *A. niger* was grown for nine days was extracted with ethyl acetate. Fractionation of the ethyl acetate extract, A, with hexane–acetone on silica afforded two fractions I and II. Purification of these fractions yielded compounds **1** and **2**, from fraction I and compounds **3**–**5**, from fraction II. The acidic aqueous portion (pH 2) was dried at reduced pressure and redissolved in methanol. Evaporation of the methanol and purification of the residue, gave Fraction B, which on a basic resin yielded compounds **6** and **7** identified as mannitol and citric acid, respectively. All crude and purified fractions were bioassayed on seed germination (Table 1).

Compound **1**, had no UV absorption, but showed characteristic IR bands at 3300, 1720 and 1625 cm^{-1} indicative of a hydroxyl group, an ester carbonyl and an isolated double bond, respectively. The single band in the IR spectrum at 960 cm^{-1} suggested the presence of an isolated *trans* double bond [15]. A triplet at δ 0.89 (3H), doublet at δ 1.01 (3H) and the singlet at 3.63 (3H) ppm were indicative of an ethyl, secondary methyl and methoxy groups, respectively. A 2H multiplet at 5.41 ppm supported the olefinic functionality indicated by the IR spectrum. The multiplet (1H) at 3.50 ppm was assigned to the C-H of the secondary hydroxyl, while the singlet (1H) at 2.25 ppm, which exchanged with D_2O, supported the presence of a OH group.



1



2 R^1 = H, R^2 = CH_2CH_2OH
3 R^1 = H, R^2 = CH_2COOH
4 R^1 = H, R^2 = OCH_2COOH
5 R^1 = OMe, R^2 = CH_2COOH
6 Mannitol
7 Citric acid

* Present address: 420 Plant and Soil Science, Department of Horticulture, Michigan State University, East Lansing, Michigan 48824, U.S.A.

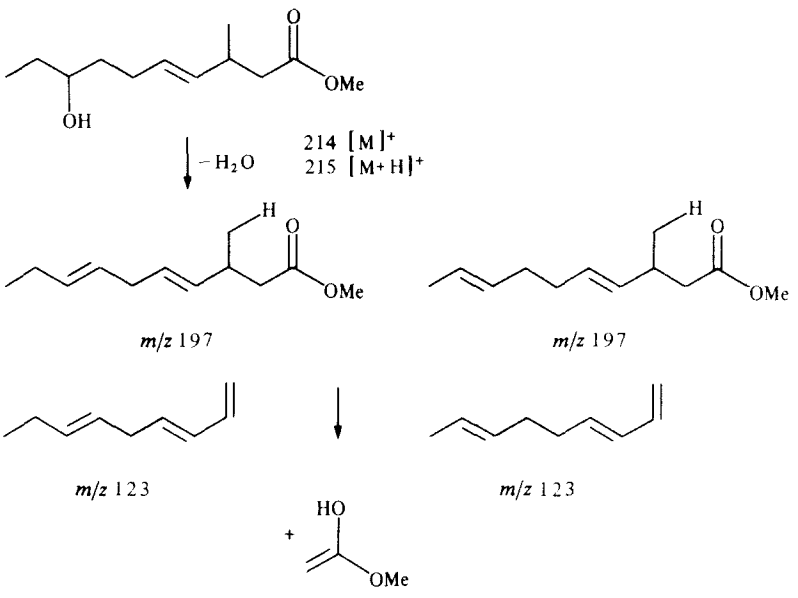
Table 1. Activity at 100 ppm of fractions A, B, I, and II and compounds **1**—**7** on seed germination

Compound	% Seed germination inhibition					
	Cress	Lettuce	Cotton	Corn	Soybean	Velvetleaf
Fraction A	90	90	60	20	60	80
Fraction B	95	95	30	10	30	40
Fraction I	80	80	0	0	0	0
Fraction II	85	85	0	0	30	60
1	80	80	0	0	0	0
2	0	0	0	0	0	0
3	95	90	10	0	20	40
4	90	90	0	0	0	30
5	95	95	35	20	40	45
6	0	0	0	0	0	0
7	95	95	0	0	0	0

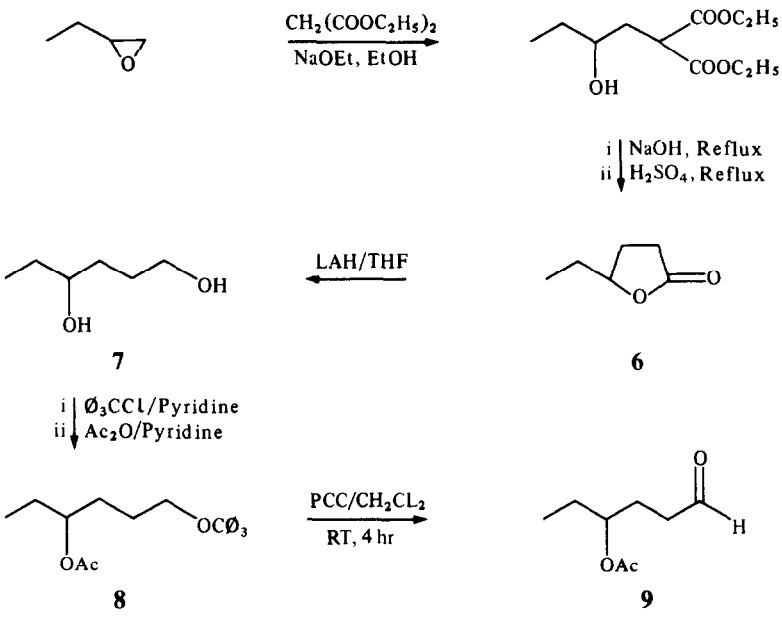
These pieces of evidence were linked to a single structure for compound 1 through a ^{1}H NMR decoupling experiment. The multiplet (1H) at 2.62 ppm was affected when the signals at 1.01 and 2.26 ppm were independently irradiated. Similarly, irradiation of the multiplet (1H) at 2.62 ppm changed the multiplet (2H) at 2.26 ppm to an AB pattern and the doublet (3H) at δ 1.01 to a singlet. It also changed half the multiplicity at δ 5.41 (2H) for the C_4-C_5 olefinic protons to a doublet at δ 5.33. Decoupling the signal at 2.02 ppm also affected the olefinic protons in the similar manner to give a doublet at 5.50 ppm. This result indicated that the protons at C-4 appear at 5.33 and C-5 at 5.50 ppm, respectively. The only other significant effect of irradiation was on the signal at 1.45 ppm. The chemical shift and the decoupling data confirmed the presence of CH=CH–CH(Me)—CH_2COOMe moiety in compound **1**. decoupling the olefinic signals at 5.41 ppm and the multiplet at 3.50 ppm showed effects only on the multiplets at 2.62, 2.02 ppm, and 1.45 ppm, respectively.

The EI mass spectrum of compound **1** gave the highest mass peak at *m/z* 196 and DCI mass showed the $[M+H^+]$ ion at *m/z* 215. The base peak at *m/z* 123 in the DCI mass spectrum can be explained by the loss of H_2O and $C_3H_6O_2$ from the $[M+H]^+$ (Scheme 1). The proton decoupled experiment along with ^{13}C NMR and mass spectral fragmentation of compound **1** proved its proposed structure.

Further confirmation of the structure of compound **1** was obtained by the chemical synthesis (Schemes 2, 3). One pot synthesis of lactone **6** was carried out in high yield by reacting 1, 2-epoxybutane with diethylmalonate anion (Scheme 2) and refluxing the product first with aqueous sodium hydroxide and then with sulphuric acid. The lactone **6** was converted to hexane-1, 4-diol, **7**, by lithium aluminium hydride in THF. The monotrityl derivative of the diol was acetylated to yield compound **8**, which was then oxidized to aldehyde **9**. Formation of the aldehyde without first isolating the detritylated alcohol



Scheme 1.



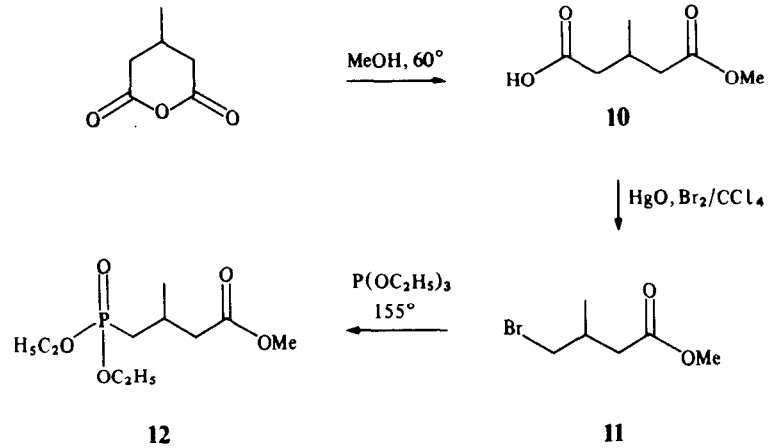
Scheme 2.

acetate is a novel and efficient procedure. The aldehyde was formed in high yield (93%) by reacting **8** with PCC in methylene dichloride with acetic acid as catalyst at room temperature. This eliminates the intermediate step for the removal of the trityl group to produce the primary alcohol prior to the oxidation to the aldehyde. The use of PCC in methylene dichloride with acid catalyst could be used as a general procedure to produce aldehydes from primary alcohols on occasions where other hydroxy protecting groups such as acetates are present.

Heating methylglutric anhydride in dry methanol at 60° for 1 hr gave the monomethyl ester, **10**, which on Hunsdiecker reaction [16] with red mercury oxide and bromine in tetrachloromethane afforded the brominated methyl ester **11**. An attempt to prepare the phenylphosphonium bromide of **11** with triphenyl phosphine was not successful. Hence, compound **11** was reacted with triethyl phosphite to produce the phosphonate, **12** (Scheme 2). The final step, the Witting reaction [17, 18] of compounds **9** and **12** was carried out in monoglyme and potassium hydride. The overall yield of product **1** was low. However, the synthetic fatty acid methyl ester was identical to the natural product **1**, by comparison of spectral data. The yield of Wittig reaction may be improved by proper manipulation of the reaction conditions.

Phenylethanol, **2**; phenylacetic acid, **3**; phenoxyacetic acid, **4**; *p*-methoxyphenylacetic acid, **5**; mannitol, **6** and citric acid, **7**, were also isolated and identified by spectral methods.

Antifungal bioassay of all compounds as performed using *Cladosporium herbarum*. TLC plates with doses of test compounds of 5 μg were sprayed with *C. herbarum* [19]; zones of inhibition were detected for compounds **2**–**5**. Compound **1** did not show any antifungal activity. All compounds, except **6**, inhibited cress and lettuce seed germination (Table 1). A fatty acid methyl ester with a similar structure to **1** isolated from *Hibiscus* bark was also reported to inhibit lettuce seed germination [20]. Phytotoxins isolated from *Rhizoctonia solani* [21], *m*-hydroxy and *m*-methoxyphenylacetic acids and various other hydroxy and methoxyphenylacetic acids were studied for



their plant growth regulatory effects [21]. Our whole plant experiment using fraction I, containing compounds **1** and **2**, fraction II, containing compounds **3**–**5**, and fraction B, containing **6** and **7** on corn, cotton, soybean and velvetleaf did not show much activity on their growth and flowering although fraction II provided variable results on velvetleaf. Fraction II produced a decrease (15%) on the flowering and seed production of velvetleaf. This fraction also had a slight effect on early growth of cotton and soybean. The most sensitive plant was velvetleaf. We did not study the effect of individual compounds on the above test plants since fractions I, II and B did not show significant activity.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.45 MHz): $CDCl_3$. MS (70 eV, EI; DCI, butane); UV: MeOH; IR: $CHCl_3$. Prep. TLC and TLC were carried out on silica gel and HPLC on C-18, MeOH–H_2O solvent systems.

Aspergillus niger var. Tieghem was isolated from decaying plantlets of *Kalanchoe daigremontiana* and identified by Dr Z. Lawrence (Commonwealth Mycological Institute, Kew) (IMI # 297005). The fungus was grown on shake bacto potato dextrose broth (PD, 24g/l, final pH = 5.65 ± 0.2 at 25°, 90 rpm, 28°, 9 days).

Germination inhibition. Seeds (20) were placed on filter paper in a 5 cm Petri dish with 1 ml test soln containing 100 ppm test compounds per dish; control seeds were treated with H_2O alone. All dishes were placed in a moist plastic box and kept in the dark at ambient temp (72 hr). The expt was repeated × 3 and the results represent germination of seeds expressed as a % of the controls.

Antifungal bioassay. Solns of test compounds (5 μg) were spotted on TLC plates and air-dried. *C. Herbarum* spores were suspended in a soln of D(+) glucose (1.5 g) in Homan's soln (30 ml), made by dissolving KH_2PO_4 (7 g), $NA_2HPO_4{\cdot}2H_2O$ (3 g), KNO_3 (4 g), $MgSO_4{\cdot}7H_2O$ (1 g) and NaCl (1 g) per l of H_2O, and sprayed uniformly on the TLC plate with test compounds. The plates were incubated in a moist atmosphere at 28° (72 hr).

Whole plant experiment. Plants (at 4 leaf stage) were sprayed with test solns of fractions in 2% DMSO in H_2O and kept in a growth chamber at 25°, subjected to 16:8 hr light-dark photoperiod. After 3 weeks the fr. wt and dry wts were determined and the results represent the fr. wt/dry wt % variations to the control.

Extraction and isolation of compounds. Six 1 l. Erlenmeyer flasks containing potato dextrose (PD) broth (500 ml) were inoculated with spores of *A. niger* previously grown on solid PDA plates. The cultures were grown on a rotary shaker at 28°. On day 9, the mycelium was filtered from the broth and the clear broth (2.8l) extracted with EtOAc (4 × 500 ml). The EtOAc extract, evapd *in vacuo*, afforded fraction A (2.07 g). The aq. portion was dried at red. pres., dissolved in MeOH and filtered. The MeOH soln was then evapd *in vacuo* giving fraction B (55 g).

The crude EtOAc portion, fraction A, brown oily residue (690 mg) was fractionated by silica gel flash CC with hexane–Me_2CO mixts and produced two fractions, I and II. Fraction I, a yellow fragrant oil (180 mg), was further purified by HPLC on a C-18 reverse phase column, MeCN–H_2O (4:1). This gave compounds **1** (10 mg) and **2** (60 mg). Fraction II, a brown residue (350 mg), purified by extensive TLC and HPLC gave the pure compounds **3** (28 mg), **4** (40 mg) and **5** (140 mg). The MeOH-soluble portion, Fraction B (2 g), was purified on an Amberlite CG-400, a strongly basic resin column. The pre-washed resin (70 g) was packed in MeOH–H_2O (1:1). After application of fraction B, three bed vols of eluting solvent were collected. The column was then eluted with 3M HCl in MeOH (2 bed vols). The neutral portion, was evapd to dryness and recrystallized from H_2O–MeOH, gave needle-like crystals of compound **6** (200 mg) and the acidic portion on drying and recrystallization from MeOH–hexane yielded needles of compound **7** (1.1 g).

Compound **1**. Colourless, oily, $C_{12}H_{22}O_3$ (M_r 214); no UV absorption, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH), 1720 (ester), 1625 (C = C), 960 (isolated *trans* double bond); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.41 (2H, *m*, C-4, C-5), 3.63 (3H, *s*, OMe), 3.50 (1H, *m*, C-8), 2.62 (1H, *m*, C-3), 2.26 (2H, *m*, C-2), 2.25 (1H, *s*, exchanged with D_2O, OH), 2.06 (2H, *m*, C-6), 1.45 (4H, *m*, C-7, C-9), 1.01 (3H, *d*, *J* = 7Hz, Me), 0.89 (3H, *t*, *J* = 7Hz, C-10); ^{13}CNMR ($CDCl_3$,75.45 MHz): δ 173.13 (*s*, C = O), 134.56 (*d*, C-4), 129.16 (*d*, C-5), 76.47 (*d*, C-8), 51.78 (*q*, OMe), 41.75 (*t*, C-2), 36.45 (*t*, C-6), 33.71 (*d*, C-3), 30.18 (*t*, C-9), 28.82 (*t*, C-7), 20.43 (*q*, CH_3), 9.91 (*q*, C-10); MS (DCI) *m/z* (rel.int.): 215 $[M+H]^+$ (25), 197 ($[M+H^+]-H_2O$) (55), 165 ($[M+H]^+ - [H_2O+MeOH]$) (60), 123 ($[M+H]^+ - [H_2O+C_3H_6O_2]$) (100), 81 (55); MS (EI) *m/z* (rel. int.): 196 $[M-H_2O]^+$ (15), 165 ($M^+ - [H_2O+OMe]$) (25), 153 ($M^+ - [H_2O+MeCO]$) (38), 140 (85), 81 (100).

Compound **2**. Colourless fragrant oil, $C_8H_{10}O$ (M_r 122); ^{1}H NMR ($CDCl_3$): δ 2.80 (2H, *t*, *J* = 6 Hz, benzylic), 3.85 (2H, *t*, *J* = 6 Hz, OCH_2), 7.25 (5H, *s*, aromatic); ^{13}C NMR ($CDCl_3$): δ 39.62 (*t*, CH_2 benzylic), 63.78 (*t*, CH_2O), 126.75 (*s*), 128.90 (*d*), 129.51 (*d*), 140.0 (*d*) all aromatic. Spectral data is identical to a commercial sample of phenylethanol.

Compound **3**. Colourless oil, $C_8H_8O_2$ (M_r 136); ^{1}H NMR ($CDCl_3$): δ 3.60 (2H, *s*, CH_2), 7.30 (5H, *s*, aromatic), 11.80 (1H, *br*, exchanged with D_2O, acid); ^{13}C NMR ($CDCl_3$): δ 40.01 (*t*, CH_2), 179.80 (*s*, acid), 142.0 (*d*), 130.20 (*d*), 129.80 (*d*), 127.20 (*s*), all aromatic. Spectral data is identical to a commercial sample of phenylacetic acid.

Compound **4**. Colourless solid, $C_9H_{10}O_3$ (M_r 166), mp 87–89°; ^{1}H NMR ($CDCl_3$): δ 3.53 (2H, *s*, benzylic), 3.67 (3H, *s*, OMe), 6.76 (2H, *d*, *J* = 8 Hz, 3-H, 5-H), 7.10 (2H, *d*, *J* = 8 Hz, 2-H, 6-H); ^{13}C NMR ($CDCl_3$): δ 40.47 (*t*, CH_2), 55.49 (*q*, OMe), 114.4 (*d*), 123.8 (*s*), 130.81 (*d*), 160.0 (*s*), all aromatic, 180.0 (*s*, acid). Spectral data identical to commercial phenoxyacetic acid.

Compound **5**. Colourless solid, $C_8H_8O_3$ (M_r 152), mp 97–98°; ^{1}H NMB ($CDCl_3$): δ 3.60 (3H, *s*, OMe) 4.30 (2H, *s*, CH_2), 7.10 (5H, *m*, aromatic), 12.01 (1H, *s*, exchangeable with D_2O). Identical to commercial *p*-methoxyphenylacetic acid.

Synthesis of compound **1**. *Hexane-1,4-diol* **7**. Metallic Na (2.4 g) were added to EtOH (250 ml). When all the Na had dissolved, diethylmalonate (11.32 ml, 16 g) was added and allowed to react (5 min). 1,2-Epoxybutane (7.2 g, 5.21 ml) was then introduced into the reaction mixt and stirred at room temp. (6 hr). Excess EtOH was removed *in vacuo* and aq. NaOH soln (10 M, 50 ml) added and the mixt refluxed (2 hr). The reaction mixt was cooled, acidified with H_2SO_4 (50%) and a further 80 ml of acid added before refluxing (24 hr). The reaction mixt was then extd with Et_2O after neutralizing with solid Na_2CO_3. The Et_2O layer was dried ($MgSO_4/Na_2CO_3$) and evaporation to dryness *in vacuo* afforded a colourless liquid, **6**, 7.25 g; ^{1}H NMR ($CDCl_3$): δ 4.37 (1H, *m*, CH-O), 2.47 (1H, *m*, CH_2CO), 2.26 (1H, *m*, CH_2CO), 1.78 (4H, *m*, $CH_2 \times 2$), 0.93 (3H, *t*, *J* = 6 Hz, Me); ^{13}C NMR ($CDCl_3$): δ 176.09 (*s*, C=O), 81.06 (*d*, CH–O), 27.76, 27.43, 26.45 (all *t*, CH_2), 8.46 (*q*, Me); MS *m/z* (rel. int.): 114 $[M]^+$ (100), 97 $[M-OH]^+$ (40), 85 (95), 67 (75), 55 (100). The above lactone was then treated with LAH (1 g) in dry THF (200 ml) at room temp (2 hr). Excess LAH was removed by reaction with EtOAc and the reaction mixt. evapd to dryness. The residue was then extracted with $CHCl_3$ and the $CHCl_3$ soln after evaporation at red. pres. yielded a viscous colourless liquid, hexane-1,4-diol, **7**, (5.7 g); ^{1}H NMR ($CDCl_3$): δ 3.8 (2H, *m*, H-1 × 2), 3.6 (1H, *m*, H-4), 3.0 (2H, *br s*, exchangeable with D_2O, OH), 1.7 (3H, *m*) 1.5 (3H, *m*),

0.94 (3H, *t*, $J=6$ Hz, Me); ^{13}C NMR ($CDCl_3$): δ 72.14 (*d*, C-4), 61.66 (*t*, C-1), 32.83, 29.35, 28.15 (all *t*, C-2, C-3, C-5), 9.15 (*q*, C-6). MS *m/z* (DCI) (rel. into) 118 $[M]^+$ (60), 86 $[M-2H_2O]^+$ (400).

Compound **9**. Diol, **7**, (4.76 g) was dissolved in pyridine (30 ml) and trityl chloride (12.37 g) added. The reaction mixt was heated to 60° (2 hr) and excess pyridine was removed under red. pres. The mixt. thus obtained was then extracted with hexane. The hexane extract, containing trityl and some ditrityl impurities, was dried *in vacuo* and purified by flash CC (silica gel, hexane–Me_2CO, 3:1). The monotrityl derivative thus obtained (11 g) was a colourless viscous oil; ^{1}H NMR ($CDCl_3$): δ0.90 (3H, *t*, $J=6$ Hz, Me), 1.45 (3H, *m*), 1.70 (3H, *m*), 2.80 (1H, *s*, exchanged with D_2O, OH), 3.10 (2H, *t*, $J=6.5$ Hz, CH_2-O-trityl), 3.50 (1H, *m*, CH-O), 7.3 (15H, *m*, trityl). This was acetylated with Ac_2O (3 ml) in pyridine (20 ml) at room temp (18 hr). Excess Ac_2O and pyridine from the reaction mixt gas removed under red. pres. and the product, **8**, was stirred with PCC (1 mol excess) in CH_2Cl_2 (200 ml) in the presence of a catalytic amount of HOAc at room temp (4 hr). The brown soln obtained was passed through a column of silica gel and the colourless CH_2Cl_2 soln was evapd to dryness. Further purification of this product by silica gel CC (hexane) afforded a colourless liquid, 9, (3.0 g), $C_8H_{14}O_3$ (M_r 158), ^{1}H NMR ($CDCl_3$): δ 9.78 (1H, *s*, not exchangeable with D_2O, CHO), 4.85 (1H, *m*, H-4), 2.35 (2H, *t*, $J=6$ Hz), 2.0 (3H, *s*, acetate), 1.85 (2H, *m*), 1.6 (2H, *m*), 0.90 (3H, *t*, $J=6$ Hz, Me); ^{13}C NMR ($CHCl_3$): δ 9.34 (*q*, Me), 20.88 (*q*, Me), 25.64 (*t*, CH_2), 26.80 (*t*, CH_2), 39.69 (*t*, CH_2), 74.40 (*d*, O-CH), 170.38 (*s*, C=O), 201.01 (*d*, CHO).

Compound **10**. 3-Methylglutaric anhydride (Aldrich) (10 g) was dissolved in dry MeOH and kept at 50° (1.5 hr). The MeOH was evapd and the product distilled at red. pres. The fraction collected at 172–173° (10–12 mm Hg) was a colourless liquid, **9**. (9 g), $C_7H_{12}O_4$ (M_r 160). ^{1}H NMR ($CDCl_3$): 11.30 (1H, *br s*, exchangeable with D_2O, acid), 3.54 (3H, *s*, OMe), 2.40 (2H, *m*), 2.22 (2H, *m*), 1.01 (3H, *d*, $J=7$ Hz, Me); MS (DCI) *m/z* (rel. int.): 143 $[M-OH]^+$ (92), 129 $[M-OMe]^+$ (100), 114 (35), 101 (48).

Compound **11**. The mono Me ester of 3-methylglutaric acid, **10** (8 g) was dissolved in dry CCl_4 (200 ml). Red HgO (9.4 g) was heated until it became bright red and then it was poured into the CCl_4 soln of **9** and refluxed (5 min). A soln of Br_2 in CCl_4 (2.4 ml/50 ml) was added to the refluxing mixt slowly (15 min) in the dark and refluxing continued (1.5 hr) in the dark. Excess HgO and $HgBr_2$ were filtered off from the cooled mixt. and the colourless soln stirred with dry K_2CO_3 (5 min) and filtered. The filtrate was evapd to dryness *in vacuo* to afford a colourless liquid, **11**, (8 g), $C_6H_{11}O_2Br$ (M_r 194, 196); ^{1}H NMR ($CDCl_3$): δ 3.55 (3H, *s*, OMe), 3.32 (2H, *m*, 2 × H-4), 2.40 (1H, *m*, H-2), 2.18 (2H, *m*, H-2, H-3), 0.97 (3H, *d*, Me); ^{13}C NMR ($CDCl_3$): δ 171.87 (*s*, C=O), 51.23 (*q*, OMe), 39.75 (*t*, CH_2), 38.66 (*t*, CH_2), 31.78 (*d*, CH), 18.46 (*q*, Me); MS (DCI) *m/z* (rel. int): 197 (10), 195 (15) both $[M+H]^+$, 165 (20), 163 (25), both $[M+H]^+-MeOH$], 115 (30) $[M-Br]^+$, 74 (100).

Compound **12**. The bromide, **10** (5 g) was added to preheated $P(OEt)_3$ (6 ml) under Ar and heating continued at 155° (6 hr). The cooled reaction mixt was purified on a short column of neutral Al_2O_3 (hexane) and the resulting product was a colourless liquid, **12** (4.8 g), $C_{10}H_{21}O_5P$ (M_r 252); ^{1}H NMR ($CDCl_3$): 4.10 (4H, *m*, OCH_2 × 2), 3.65 (3H, *s*, OMe), 2.40 (3H, *m*), 1.75 (2H, *m*), 1.30 (6H, *t*, $J=6$ Hz, Me × 2), 1.10 (3H, *d*, $J=6$ Hz, Me), ^{13}C NMR ($CDCl_3$): δ 171.80 (*s*, C=O), 74.0 (*t*), 63.0 (*t*), 51.60 (*q*, OMe), 40.01 (*t*), 31.20 (*d*), 18.0 (*q*), 16.65 (*q*), MS (DEI) (rel. int.): 252 $[M]^+$ (5), 221 $[M-OMe]^+$ (15), 193 (10), 179 (20), 152 (25), 137 (60), 114 (65), 109 (100), 81 (48), 73 (30).

Synthetic compound **1**. The phosphonate, **12** (Hg) and KH (250 mg) in monoglyme (5 ml) were stirred at room temp (2 hr). To this yellow soln, compound **8** (650 mg) in monoglyme (2 ml) was introduced and stirred (6 hr) under Ar at room temp. The mixt. was then acidified with HOAc, dil with H_2O and extd with EtOAc. The aq layer was dried, treated with KOH in MeOH (5%) (4 hr at room temp), acidified with HCl inn MeOH (3 M) and evapd to dryness. The residue thus obtained was dissolved in $CHCl_3$, filtered and the filtrate reacted with CH_2N_2 in Et_2O. The soln was then evapd *in vacuo* and purified by HPLC, MeCN-H_2O (4:1) on a C-18 reverse phase column. The fraction collected, which had the same R_f as the natural product, **1**, was a colourless oily compound (60 mg) with identical IR, ^{1}H NMR, ^{13}C NMR and MS data.

REFERENCES

1. Nair, M. G., Epp, M. D. and Burke, B. A. (1988) *J. Chem. Ecol.* **14**, 585.
2. Moyer, A. J. (1953) *Appl. Microbiol.* **1**, 1.
3. Walksman, S. A. and Karow, E. O. (1947) *Ind. Eng. Chem.* **39**, 821.
4. Das, A. and Roy, P. (1980) *Adv. Biotech.* **1**, 51.
5. Cutler, H. G., Crumleus, F. C., Cox, R. H., Hernandes, O., Cole, R. J. and Dorner, J. W. (1979) *J. Agric. Food Chem.* **7**, 592.
6. Gorst-Allman, C. P., Steyn, P. S. and Rabie, C. J. (1980) *J. Chem. Soc. Perkin Trans. I* 2474.
7. Tanaka, H., Wang, P. L. and Namiki, M. (1972) *Agric. Biol. Chem.* **36**, 2511.
8. Ghosal, S., Biswas, K. and Chakrabarti, D. K. (1979) *J. Agric. Food Chem.* **27**, 1347.
9. Nagasawa, H., Isogai, A., Ikeda, K., Sato, S., Murakoshi, S., Suzuki, A. and Tamara, S. (1975) *Agric. Biol. Chem.* **39**, 1901.
10. Anderegg, R. J., Biemann, K., Buchi, G. and Cushman, M. (1976) *J. Am. Chem. Soc.* **98**, 3365.
11. Kobbe, B., Cushman, M., Wogan, G. N. and Demain, A. L. (1977) *Appl. Environ. Microbiol.* **33**, 996.
12. Isogai, H., Horii, T., Zuzuki, A., Murakoshi, S., Ikeda, K., Sato, S. and Tamara, S. (1975) *Agric. Biol. Chem.* **39**, 739.
13. Rabachi, M., Newmann, J. and Lavellay, J. (1974) *Phytochemistry* **13**, 637.
14. Laine, R. A., Griffin, P. F. S., Sweeley, C. C. and Brennan, P. J. (1972) *Biochemistry* **11**, 2267.
15. Gunstone, F. D. (1967) *An Introduction to the Chemistry and Biology of Fatty Acids and their Glycosides*, p. 43. Chapman & Hall, London.
16. Cristol, S. J. and Firth Jr., W. C. (1961) *J. Org. Chem.* **29**, 1279.
17. Davis, J. B., Jackman, L. M., Siddons, P. T. and Weedon, B. C. L. (1966) *J. Chem. Soc. (C)* 2154.
18. House, H. O., Jones, V. K. and Frank, G. A. (1964) *J. Org. Chem.* 3327.
19. Homans, A. L. and Fuchs, A. (1970) *J. Chromatog.* **51**, 327.
20. Nakatini, M. N., Fukunaga, Y. and Hase, T. (1986) *Phytochemistry* **25**, 449.
21. Mandava, N. B., Orellana, R. G., Warthen, J. D., Worley, J.F., Dutky, S. R., Finegold, H. and Weathington, B. C. (1980) *J. Agric. Food Chem.* **28**, 71.