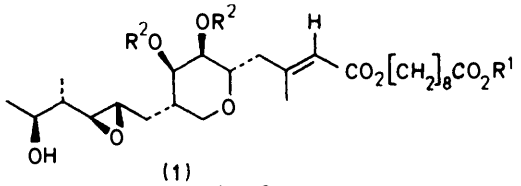
The Chemistry of Pseudomonic Acid. Part 2.[1] The Conversion of Pseudomonic Acid A into Monic Acid A and its Esters

By **John P. Clayton,* Kong Luk,** and **Norman H. Rogers,*** Beecham Pharmaceuticals, Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ

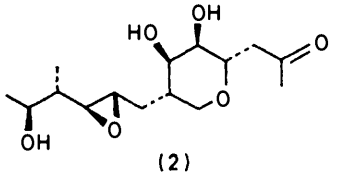
By suitable protection and deprotection, the 9-hydroxynonanoic acid side-chain of pseudomonic acid A (1a), a naturally occurring antibiotic, was cleaved in a highly efficient one-pot reaction to the allylic acid (3a), 4-[5*S*-(2*S*,3*S*-epoxy-5*S*-hydroxy-4*S*-methylhexyl)-3*R*,4*R*-dihydroxytetrahydropyran-2*S*-yl]-3-methylbut-2(*E*)-enoic acid. We have ascribed the trivial name, monic acid A, to this allylic acid. Esters of monic acid A were readily prepared from the free acid (3a) and also from the ketone (2) which could be condensed with phosphonoacetates.

PSEUDOMONIC ACID A, a naturally occurring antibiotic produced by fermentation of a strain of Pseudomonas fluorescens, was assigned the gross structure (1a) by

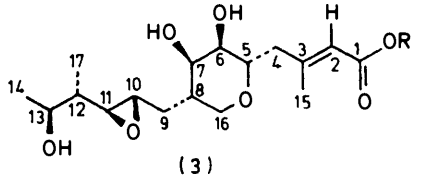


(1)

a; $R^1 = R^2 = H$
b; $R^1 = Me, R^2 = H$
c; $R^1 = Me, R^2 = Me_2C$



(2)



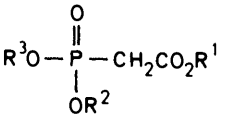
(3)

a; R = H
b; R = CH_3
c; R = C_2H_5
d; R = CH_2Ph

Chain and Mellows.[2] In a previous paper we described the preparation of the ketone (2) derived from pseudomonic acid A by ozonolysis.[1] An *X*-ray analysis of a heavy atom derivative of (2) indicated the absolute stereochemistry at each of the eight chiral centres to be that illustrated.

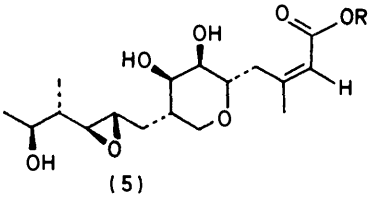
Pseudomonic acid A is highly bound to human serum protein to the extent of 95%. In a programme of work to explore the effect of structural changes on biological properties we were concerned to reduce the degree of binding since it is only the unbound portion of an antibiotic that is free to exert its antibacterial action. Pseudomonic acid A may be described as an ester of an allylic acid (3a) and 9-hydroxynonanoic acid. Since in general protein binding appears to correlate with lipophilicity one approach to reducing the extent of binding was to attempt the replacement of this C-9 acidic alcohol with shorter chain alcohols. In other words, we sought to effect a transesterification of pseudomonic acid A (1a).

In our first approach to simple esters of the allylic acid (3a), to which we have given the trivial name monic acid A, we utilised the readily available ketone triol (2). Reaction of (2) with the Wadsworth–Emmons reagent, triethyl phosphonoacetate (4a), in a stirred suspension of sodium hydride in tetrahydrofuran, provided a complex mixture in which both ethyl monate A (3c) and ethyl isomonate A (5a) were present as shown by h.p.l.c.

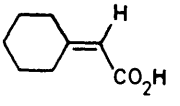


(4)

a; $R^1 = R^2 = R^3 = Et$
b; $R^1 = R^2 = R^3 = Me$
c; $R^1 = CH_2Ph, R^2 = R^3 = Et$
d; $R^1 = CH_2CCl_3, R^2 = R^3 = Et$
e; $R^1 = SiMe_3, R^2 = R^3 = Et$
f; $R^1 = H, R^2 = R^3 = Et$
g; $R^1 = R^3 = Et, R^2 = H$



(5)



(6)

a, R = Et
b, R = Me
c, R = $[CH_2]_8CO_2Me$

analysis. The total yield of the two isomers, *ca.* 15%, was increased to 80% when the experiment was repeated with prior protection of the hydroxy groups in the ketone triol (2). This was achieved by pretreatment of (2) with *NO*-bistrimethylsilylacetamide. Chromatography of the crude product from this reaction afforded the

crystalline *E*-isomer (3c), m.p. 99—100°, and the oily *Z*-isomer (5a) in a ratio of *ca.* 3 : 1. We had previously shown that methyl pseudomonate A (1b) the natural *E*-isomer, could be readily distinguished from its *Z*-isomer, methyl isopseudomonate A (5c), by a comparison of the shifts of the vinylic methyl groups in both the ^1H and ^{13}C n.m.r. spectra.[1] A similar application readily distinguished the monate esters (3c) and (5a). The ^1H n.m.r. shift of the vinylic methyl group was δ 2.19 in (3c), consistent with a deshielding effect of the *cis*-oriented ester group, and δ 1.98 in (5a) in which the deshielding effect was absent. Further evidence for these assignments came from a study of the nuclear Overhauser effects for the two geometrical isomers. An 18.9% enhancement of the vinylic proton signal was observed on irradiation of the vinylic methyl group in (5a) whereas no enhancement occurred in the case of (3c). Condensation of the trimethylsilyl-protected ketone (2) with triethyl phosphonoacetate (4b) afforded methyl monate A (3b), m.p. 124—125°, and methyl isomonate A (5b) in a ratio of 3 : 1 and a combined yield of 55%, and in a similar reaction benzyl *PP*-diethyl phosphonacetate (4c) afforded benzyl monate A (3d) as an oil in 17% yield.

Clearly a highly desirable intermediate in the preparation of esters of monic acid A was monic acid A (3a) itself. We therefore turned our attention to condensations of ketone (2) with phosphonoacetates which incorporated a chemically labile ester group. The availability of *PP*-diethylphosphonoacetic acid (4f), see below, enabled us to prepare trichloroethyl (4d) and trimethylsilyl *PP*-diethylphosphonoacetate (4e). However when these phosphonoacetates were condensed with the protected ketone (2) we were unable to detect any of the desired products.

PP-Diethylphosphonoacetic acid (4f) has not to our knowledge been used in a Wadsworth–Emmons reaction. Since a successful condensation of (4f) with ketone (2) would lead directly to monic acid A (3a) we prepared (4f) from ethyl *PP*-diethylphosphonoacetate (4a) by hydrolysis with one equivalent of sodium hydroxide solution.[3] In contrast to previous attempts,[4] this procedure provided (4f) in 61% yield as an oil, which was readily distinguished from the isomeric ethyl *P*-ethylphosphonoacetate (4g) on the basis of its ^1H n.m.r. spectrum. The spectrum of (4f) exhibited an eight line signal for the four methylene protons at δ 4.16 which collapsed to a doublet (J_{HP} 8 Hz) when the six proton methyl triplet at δ 1.30 was irradiated.

In a model reaction the dianion of *PP*-diethylphosphonoacetic acid (4f) generated with two equivalents of sodium hydride in dimethylformamide (DMF) was condensed with cyclohexanone. A quantitative yield of cyclohexylideneacetic acid (6), m.p. 88—89° (lit.,[5] 89—90°), was obtained. However, when this procedure was applied to the trimethysilyl-protected ketone (2) a multicomponent mixture resulted, in which a very small amount of monic acid A (3a) was demonstrated by treatment of the product with diazomethane and h.p.l.c. comparison of the esterified complex with authentic methyl monate A (3b).

It was clear from the above results that the ketone (2) was not a satisfactory precursor to esters of monic acid A or to the acid itself. Yields were variable, mixtures of geometrical isomers were formed, and in our hands certain phosphonoacetates failed to react. We therefore turned our attention to studying the direct hydrolysis of pseudomonic acid A (1a) to monic acid A (3a). Chain and Mellows had attempted to cleanly remove the 9-hydroxynonanoic acid group by treating methyl pseudomonate A (1b) with methanolic potassium hydroxide.[2] They found that while the allylic ester bond was hydrolysed under these conditions concomitant rearrangement of the rest of the molecule took place involving loss of the epoxide grouping. The products of this rearrangement were not characterised. In our first experiments pseudomonic acid A (1a) was treated at pH 11.6 at room temperature overnight and with potassium hydrogencarbonate in refluxing methanol. In both cases loss of the epoxide grouping was observed and 9-hydroxynonanoic acid was identified in the hydrolysis products. However hydrolysis of (1a) by aqueous potassium carbonate at 60° did produce a very small amount of monic acid A (3a) together with rearranged products as indicated by h.p.l.c. analysis of the methylated mixture.

Our attempts to effect a transesterification of (1a) with methanol were likewise unsatisfactory. When the sodium salt of (1a) was refluxed in methanol with potassium cyanide as catalyst the complex mixture provided no evidence of the presence of methyl monate A (3b).[6] A slightly more encouraging result was obtained when methyl pseudomonate A (1b) was refluxed in anhydrous methanol containing a trace of freshly prepared sodium methoxide. A small amount of methyl monate A (3b) was formed as indicated by h.p.l.c. analysis, but was insufficient to be isolable.

We considered that while methyl monate A (3b), prepared from ketone (2) as described above, was less readily available than the natural metabolite (1a) it might prove more amenable to hydrolysis to monic acid A (3a). We therefore subjected methyl monate A (3b) to a variety of hydrolytic procedures including sodium hydroxide–sodium hydrogencarbonate in aqueous methanol at pH 11, potassium carbonate in aqueous methanol, potassium hydrogencarbonate in boiling aqueous methanol in a carbon dioxide atmosphere, boron trichloride in methylene chloride,[7] and lithium iodide in DMF in the absence and in the presence of potassium cyanide.[8] In no case was more than a trace amount of monic acid A (3a) formed.

In view of the reactive functionalities in the pseudomonic acid A molecule, the failure of the above attempts to directly convert it into monic acid A was hardly surprising. It was apparent from the nature of the degradation products * that if this approach were to

* These products have been fully characterised and will be described later.

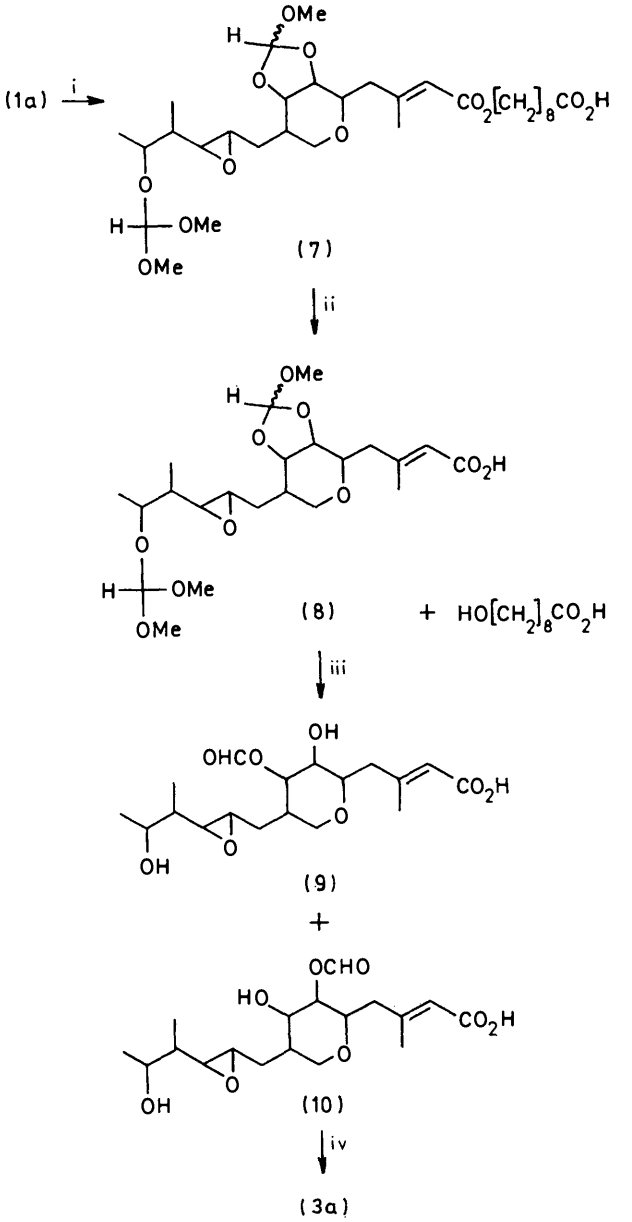
succeed it would be necessary to suitably protect the glycol system in (1a). The oily acetonide (1c) was therefore prepared as previously described from methyl pseudomonate A (1b) [2] or directly from the free acid (1a). Prior to attempting removal of the C_9 alcoholic unit from the acetonide (1c), the molecule was studied in order to define the acidic conditions required for the regeneration of the glycol system. It was found that the degree of acidity necessary was too severe and hydrolysis of the acetonide function was accompanied by extensive rearrangement. This result therefore precluded the isopropylidene group for our purpose.

Orthoesters, the preparation of which has recently been reviewed,[9] have been used in the protection of *cis*-glycol functions for example in ribonucleosides.[10,11] Orthoformates are stable in alkaline media but undergo cleavage to formate esters under mild acidic conditions. Deformylation can then be accomplished readily at pH 7 or above to generate the original glycol system. The orthoformate protecting group therefore seemed highly suited to our requirements and this indeed proved so.

In a four step, one-pot process as outlined in the Scheme pseudomonic acid A (1a) was converted into monic acid A (3a), m.p. 135—136°, in an overall yield of 78%. Initial protection of the glycol function of (1a) was achieved by acid catalysed transesterification with trimethyl orthoformate resulting in the formation of an epimeric mixture of orthoformates (7). Alkaline hydrolysis afforded the protected monic acids (8), which were not isolated, but subsequently converted into the formate esters (9) and (10) by mild acidic treatment. Deformylation to monic acid A (3a) was effected by mild alkaline hydrolysis. The intermediates shown in the Scheme are presumed since attempts to isolate and characterise them have so far proved unsuccessful. The use of h.p.l.c. to monitor the individual stages proved invaluable.

In order to establish that the monic acid A (3a) produced by the above sequence had maintained its stereochemical integrity, it was converted to its methyl ester (3b). The crystalline (3b) obtained proved identical to that derived above from ketone (2) by phosphonoacetate condensation. The excellent ^{13}C n.m.r. chemical shift correlations of methyl pseudomonate (1b), ketone (2), and methyl monate (3b) (see Experimental section) were further evidence of the chiral centres in (3a) being identical to the corresponding centres in (1b) and (2). Unequivocal proof that monic acid A (3a) possessed the stereochemistry of the natural product followed from its conversion into methyl pseudomonate A. Treatment of methyl 9-chlorononanoate with the sodium salt of (3a) afforded crystalline methyl pseudomonate A (1b), identical (m.p. and mixed m.p.) to that derived from the natural product (1a) by treatment with diazomethane.

Finally with respect to our original objective of reducing the high serum binding of pseudomonic acid A (1a), it was found that methyl monate A (3b) was significantly less protein bound to the extent of only 30%. Furthermore methyl monate A was antibacterially active with a spectrum of activity similar to that of the natural metabolite (1a). In contrast the unnatural isomers methyl isomonate (5b) and methyl isopseudomonate



SCHEME Reagents: i, $HC(OMe)_3$–*p*-$MeC_6H_4SO_3H$; ii, 5 equiv. NaOH, 65°, 3 h; iii, pH 2, 15 min, 20°; iv, pH 9.0, 3 h, 20°

(5c) were *ca.* 100-fold less active than the corresponding natural *E*-forms. Interestingly monic acid A (3a) had no antibacterial activity.

EXPERIMENTAL

M.p.s were determined on a Büchi apparatus and are uncorrected. Mass spectra were obtained at 70 eV using an A.E.I. MS9 instrument operating at 8 kV. ^1H N.m.r. data were recorded at 90 MHz on a Perkin-Elmer R32 instrument and ^{13}C measurements using a Varian CFT 20 spectrometer, both at ambient temperatures with tetramethylsilane as internal standard. The numbering system used for asigning the chemical shifts is that shown in formula (3). Column

chromatography was carried out on Merck Kieselgel H (type 60). Analytical and preparative t.l.c. were performed on pre-coated Merck Kieselgel 60 F_{254}. The analytical plates were eluted with chloroform–methanol (9 : 1 v/v) unless otherwise stated, and the components visualized by either u.v. light or charring with sulphuric acid. H.p.l.c. was performed on a Waters Associates Instrument using a C_{18} μ-Bondapak reverse phase column eluting with ammonium acetate–water–methanol buffer solutions. Both t.l.c. and h.p.l.c. were performed routinely on all compounds. Phosphonoacetates (4b and c) were prepared by standard procedures.[12] DMF was distilled *in vacuo* from calcium hydride and stored over regenerated Linde 4A molecular sieves. Acetonitrile was distilled from phosphorus pentaoxide. Tetrahydrofuran (THF) was dried over sodium and freshly distilled from lithium aluminium hydride immediately before use.

Methyl 4-[5*S*-(2*S*,3*S*-*Epoxy*-5*S*-*hydroxy*-4S-*methylhexyl*)-3R,4R-*dihydroxytetrahydropyran*-2S-*yl*]-3-*methylbut*-2(E)-*enoate* (*Methyl Monate A*) (3b) *and Methyl Isomonate A* (5b) *from Ketone* (2).—Bistrimethylsilylacetamide (5.9 ml) was added to a solution of ketone (2) (1.20 g) in acetonitrile (25 ml) at room temperature and the mixture was stirred for 1 h. The solvent was then completely evaporated *in vacuo* at 40° and the residue was dissolved in DMF (3 ml) for use in the next stage. Methyl *PP*-dimethylphosphonoacetate (3 g) in DMF (10 ml) was added dropwise over 0.5 h to a suspension of sodium hydride (80% dispersion in oil; 0.45 g) in DMF (10 ml) at 0° under nitrogen, followed by stirring at room temperature for 1 h. The solution of silylated ketone was then added dropwise over 0.5 h. to the mixture at 0° and stirring was continued at room temperature for 18 h. The mixture was poured into saturated brine (50 ml) and extracted with ethyl acetate (3 × 50 ml). The organic extract was dried and evaporated to give an oil which was dissolved in dioxan–water (4 : 1; 25 ml) and treated with hydrochloric acid (5N, 2 drops) for 10 min. Aqueous sodium hydrogencarbonate (20 ml) was then added and the mixture re-extracted with ethyl acetate (3 × 30 ml). The organic extract was dried ($MgSO_4$) and evaporated to an oil (1.2 g), which was chromatographed over silica gel (35 g). Elution of the column with chloroform–methanol (97 : 3) afforded two fractions. The first fraction was further purified by preparative t.l.c. [developed with chloroform–methanol (92 : 8)] to give *methyl isomonate A* (5b) (0.16 g) as an oil, $\nu_{max.}$ ($CHCl_3$) 1 695, 1 645, 1 220br, 1 155, 1 110, 1 080, and 1 050 cm^{-1}, $\lambda_{max.}$ (EtOH) 222 nm (ε 9 600), δ_H ($CDCl_3$) 5.87 (1 H, m, 2-H), 3.65 (3 H, s, CO_2CH_3), 2.00 (3 H, d, *J* 1 Hz, 15-CH_3), 1.20 (3 H, d, *J* 6 Hz, 14-CH_3), and 0.93 (3 H, d, *J* 6 Hz, 17-CH_3), *m/e* 359 (M^+ + 1, 1%), 358 (M^+, 0.5), 343 (0.5), 341 (0.5), 340 (1), 327 (2), 322 (2), 309 (3), 296 (2), 291 (3), 278 (8), 277 (6), 267 (5), 256 (11), 227 (100), 210 (65), and 209 (21). The second fraction afforded *methyl monate A* (3b) (0.4 g), m.p. 124—125° (from methyl acetate–hexane), $[\alpha]_D^{20}$ −11.1° (*c* 1.5 $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1 710, 1 645, 1 435, 1 220br, 1 155, 1 110, and 1 050 cm^{-1}, $\lambda_{max.}$ (EtOH) 221 nm (ε 14 700), δ_H ($CDCl_3$) 5.75 (1 H, m, 2-H), 3.65 (3 H, s, CO_2CH_3), 2.75 (2 H, m, 10- and 11-H), 2.18 (3 H, s, 15-CH_3), 1.19 (3 H, d, *J* 6.5 Hz, 14-CH_3), and 0.91 (3 H, d, *J* 7 Hz, 17-CH_3), δ_C ($CDCl_3$) 167.1 (C-1), 157.1 (C-3), 117.1 (C-2), 74.8 (C-5), 71.3 (C-13), 70.3 (C-7), 68.9 (C-6), 65.4 (C-16), 61.3 (C-11), 55.6 (C-10), 50.8 (OCH_3), 42.8 (C-4 and -12), 39.5 (C-8), 31.6 (C-9), 20.7 (C-14), 19.1 (C-15), and 12.7 p.p.m. (C-17), *m/e* 358 (M^+, 1.5%), 340 (2.5), 327 (5), 322 (3.5), 309 (4.5), 308 (3.5), 296 (5), 264 (17), 227 (100), and 209 (25) (Found: C, 60.1; H, 8.35. $C_{18}H_{30}O_7$ requires C, 60.3; H, 8.45%).

Ethyl Monate A (3c) *and Ethyl Isomonate A* (5a) *from Ketone* (2).—Bistrimethylsilylacetamide (0.75 ml) was added to a solution of ketone (2) (0.302 g) in THF (3 ml) at 0° and then stirred at room temperature for 0.5 h. The solvent was removed *in vacuo* and the residue redissolved in THF (3 ml) for use in the next stage. Ethyl *PP*-diethylphosphonoacetate (0.225 g) in THF (6 ml) was added over 0.25 h to a stirred suspension of sodium hydride (0.03 g; 80% dispersion in oil) in THF (6 ml) at 0° under nitrogen. The mixture was stirred for a further 1 h at room temperature and re-cooled to 0°. The solution of silylated ketone was added dropwise over 0.25 h to the mixture followed by warming to 60° for 0.25 h. The final mixture was poured into ice–water (9 g) and acidified to pH 2, keeping the solution homogeneous by the addition of ethanol. After 2 min aqueous sodium hydrogencarbonate (30 ml) was added and the mixture saturated with sodium chloride followed by continuous extraction with ether. The ether extract was dried ($MgSO_4$) and evaporated to an oily mixture. T.l.c. revealed two major products at R_F 0.45 and 0.40. The two components were separated by preparative layer chromatography developed three times with $CHCl_3$–MeOH (92 : 8). The less polar component (R_F 0.45), *ethyl isomonate A* (5a) (0.063 g), was obtained as an oil, $\nu_{max.}$ ($CHCl_3$) 1 690, 1 640, 1 262, 1 155, 1 085, and 1 060 cm^{-1}, $\lambda_{max.}$ (EtOH) 221 nm (ε 9 700), δ_H ($CDCl_3$) 5.93 (1 H, m, 2-H), 4.25 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), 2.06 (3 H, s, 15-CH_3), 1.30 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 1.25 (3 H, d, *J* 7 Hz, 14-CH_3), and 0.96 (3 H, d, *J* 7 Hz, 17-CH_3), *m/e* 372 (M^+, 0.5%), 354 (1), 336 (2), 327 (2), 309 (4), 291 (9), 227 (100), 224 (69), and 209 (23) (Found: C, 61.85; H, 9.2. $C_{19}H_{32}O_7$ requires C, 61.25; H, 8.65%). The more polar component (R_F 0.40), *ethyl monate A* (3c) (0.207 g), was then obtained, m.p. 99—100° (ethyl acetate–ether), $[\alpha]_D^{20}$ −1.44° (*c* 1.8, $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1 705, 1 650, 1 155, and 1 050 cm^{-1}, $\lambda_{max.}$ (EtOH) 220 nm (ε 11 100), δ_H ($CDCl_3$) 5.86 (1 H, m, 2-H), 4.23 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), 2.70—2.90 (2 H, m, 10- and 11-H), 2.26 (3 H, s, 15-CH_3), 1.30 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 1.25 (3 H, d, *J* 7 Hz, 14-CH_3), and 0.95 (3 H, d, *J* 7 Hz, 17-CH_3), δ_C ($CDCl_3$) 168.2 (C-1), 157.6 (C-3), 117.7 (C-2), 74.9 (C-5), 71.5 (C-13), 70.5 (C-7), 69.1 (C-6), 65.1 (C-16), 61.4 (C-11), 59.7 (OCH_2), 55.5 (C-10), 42.9 (C-4 and -12), 39.6 (C-8), 31.6 (C-9), 20.9 (C-14), 19.0 (C-15), and 12.8 p.p.m. (C-17), *m/e* 372 (M^+, 2%), 354 (2), 336 (3), 327 (6), 309 (7), 291 (6), 270 (11), 264 (13), 245 (10), 244 (10), 227 (100), 224 (30), and 209 (35) (Found: C, 61.1; H, 8.75%; M^+, 372.215 0. $C_{19}H_{32}O_7$ requires C, 61.25; H, 8.65%; *M*, 372.214 8).

Benzyl Monate A (3d) *from Ketone* (2).—Bistrimethylsilylacetamide (3 ml) was added to a solution of ketone (2) (0.604 g) in dry acetonitrile (10 ml) and the mixture was stirred at room temperature for 1 h. The solvent was then completely removed *in vacuo* at 40° and the residue dissolved in DMF (5 ml) for the next stage. Benzyl *PP*-diethylphosphononacetate (2.30 g) in DMF (5 ml) was added dropwise to a suspension of sodium hydride (0.240 g; 80% dispersion in oil) in DMF (5 ml) at 0° under nitrogen. The mixture was stirred under nitrogen at room temperature for 1 h. The solution of silylated ketone was then added dropwise over 0.5 h to the mixture at 0° under nitrogen, which was then stirred at room temperature for 18 h. The solution was evaporated to dryness and the residual yellow oil dissolved in ethyl acetate, washed with brine and evaporated

to an oil. The latter was dissolved in dioxan–water (4 : 1; 10 ml) and concentrated hydrochloric acid added to pH 1.5 followed by stirring at room temperature for 10 min. Excess of sodium hydrogencarbonate solution was added and the mixture was then extracted with ethyl acetate, which was washed with brine, dried ($MgSO_4$), and evaporated to an oil (1,615 g). This oil was chromatographed on silica (40 g) eluting with a gradient of methanol–chloroform (1—3%). The fractions containing pure *benzyl monate A* (by h.p.l.c. and t.l.c.) were collected and evaporated to an oil (0.150 g), $[\alpha]_D^{20}$ −5.0° (*c* 1.0 $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1 710 and 1 645 cm^{-1}, $\lambda_{max.}$ (EtOH) 219 nm (ε 14 000), δ_H ($CDCl_3$) 7.26 (5 H, s, Ph), 5.75 (1 H, m, 2-H), 5.08 (2 H, s, $PhCH_2$), 2.70 (2 H, m, 10- and 11-H), 2.18 (3 H, s, 15-CH_3), 1.17 (3 H, d, *J* 7 Hz, 14-CH_3), and 0.88 (3 H, d, *J* 7 Hz, 17-CH_3), δ_C ($CDCl_3$), 166.3 (C-1), 157.5 (C-3), 136.4, 128.5, 128.2, 128.0 (aromatic carbons), 117.3 (C-2), 74.9 (C-5), 71.3 (C-13), 70.4 (C-7), 69.0 (C-6), 65.5 (OCH_2Ph), 65.4 (C-16,) 61.3 (C-11), 55.5 (C-10), 42.8 (C-4 and -12), 39.5 (C-8,) 31.6 (C-9), 20.8 (C-14), 19.2 (C-15), and 12.7 p.p.m. (C-17) (Found: M^+, 434.229 970. $C_{24}H_{34}O_7$ requires *M*, 434.230 435).

PP-Diethylphosphonoacetic Acid (4f).—Ethyl *PP*-diethylphosphonoacetate (44.8 g) was dissolved in 1N-sodium hydroxide solution (200 ml) and the mixture was stirred for 18 h at room temperature. The pH was adjusted from 9.5 to 1.5 with dilute hydrochloric acid. The solution was saturated with sodium chloride and extracted with ethyl acetate (3 × 100 ml). The ethyl acetate layer was dried ($MgSO_4$) and evaporated to dryness *in vacuo* to give (4f) as a liquid (crystalline solid below 18°) (37.4 g, 96%), n_D^{23} 1.390 0, $\nu_{max.}$ (film) 1 730, 1 230 (P=O), 1 170, and 1 050 cm^{-1}, δ_H ($CDCl_3$) 9.35 (1 H, s, CO_2H), 4.16 (4 H, octet, CH_3CH_2OP, J_{HH} 6, J_{PH} 8 Hz), 2.98 (2 H, d, PCH_2CO_2H, J_{PH} 22 Hz), and 1.30 (6 H, t, *J* 6 Hz, CH_3CH_2) (irradiation at δ 1.30 produces a doublet at δ 4.16, J_{PH} 8 Hz) (Found: C 37.1; H, 7.05; P, 15.65. $C_6H_{13}PO_5$ requires C, 36.75; H, 6.67; P, 15.8%).

Trichloroethyl PP-*Diethylphosphonoacetate* (4d).—*PP*-Diethylphosphonoacetic acid (4f) (9.8 g) and trichloroethanol (8.22 g) were dissolved in ethyl acetate (50 ml) and cooled to 0°. Dicyclohexylcarbodi-imide (DCCI) (11.3 g) was added portionwise at 0—5°. The mixture was stirred overnight at room temperature. A small amount of acetic acid was added to destroy the excess of DCCI and after 15 min the mixture was cooled to 0° and filtered. The filtrate and washings were evaporated to dryness *in vacuo* to a liquid (17.21 g). The latter was distilled *in vacuo* to give *trichloroethyl* PP-*diethylphosphonoacetate* (4d), n_D^{23} 1.460 4, $\nu_{max.}$ (film) 2 900, 2 800, and 1 750 (C=O) cm^{-1}, δ_H ($CDCl_3$) 4.78 (2 H, s, CH_2CCl_3), 4.22 (4 H, octet, CH_3CH_2OP, J_{HH} 6, J_{PH} 9 Hz), 3.12 (2 H, d, J_{PH} 22 Hz, PCH_2), and 1.39 (6 H, t, *J* 6 Hz, CH_2CH_3) (Found: C, 29.2; H, 4.3; Cl, 32.15; P, 9.8. $C_8H_{14}Cl_3PO_5$ requires C, 29.35; H, 4.3; Cl, 32.5; P, 9.45%).

Cyclohexylideneacetic Acid (6).—Sodium hydride (0.570 g; 80% dispersion in oil) was added portionwise under dry nitrogen to a solution of *PP*-diethylphosphonoacetic acid (4f) (1.96 g) in DMF (20 ml) at 0°. After addition the mixture was stirred for a further 2 h at 0°. Cyclohexanone (0.52 ml) in DMF (15 ml) was added dropwise over 0.5 h at 0° under nitrogen. The mixture was stirred for a further 1 h at 0° and then allowed to warm to room temperature overnight. The mixture was evaporated to near dryness, diluted with excess of water and the pH taken from 12 to 1.5. The aqueous layer was extracted with ether (3 × 70 ml) and the ether washed, dried ($MgSO_4$), and evaporated to dryness to give cyclohexylideneacetic acid (6) as a crystalline solid (0.693 g, 99%), m.p. 88—89° (lit.,[5] 89—90°), $\nu_{max.}$ 1 690 (C=O) and 1 640 (C=C) cm^{-1}, δ_H ($CDCl_3$) 12.05 (1 H, s, CO_2H), 5.80 (1 H, s, =CH), 2.96 (2 H, m, α-H_2), 2.37 (2 H, m, α-H_2), and 1.70br (6 H, 3 × CH_2).

4-[5S-(2S,3S-*Epoxy*-5S-*hydroxy*-4S-*methylhexyl*)-3R,4R-*dihydroxytetrahydropyran*-2S-*yl*]-3-*methylbut*-2(E)-*enoic Acid* (*Monic Acid A*) (3a).—Pseudomonic acid A (10 g) was dissolved in trimethyl orthoformate (100 ml). Toluene-*p*-sulphonic acid (100 mg) was added and the solution stirred at room temperature for 0.5 h. The solvent was removed under reduced pressure and the residual oil immediately dissolved in 1N-sodium hydroxide solution (100 ml). The aqueous solution was stirred at 65° for 3 h, cooled, and the pH was adjusted to 7.0 with concentrated hydrochloric acid. Methanol (100 ml) was added, the pH was adjusted to 2.0 with 5N-hydrochloric acid and the solution was stirred at room temperature for 0.25 h. The pH was then raised to and maintained at 9—9.5 with sodium hydroxide solution for 3 h, when h.p.l.c. indicated complete hydrolysis of the formate. The pH was re-adjusted to 7.0. The solution was concentrated under reduced pressure to *ca.* 200 ml, saturated with sodium chloride, layered with ethyl acetate (100 ml), and acidified to pH 3.0 with stirring. The organic layer was separated and the aqueous layer further extracted with ethyl acetate (5 × 50 ml). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to yield an oil (13.8 g). Trituration with dry ether afforded *monic acid A* (3a) (3.93 g) as a crystalline solid. A further crop was obtained from the mother liquor (1.46 g). The two crops of *monic acid A* (5.39 g, 78%) were combined, m.p. 135—136°. T.l.c. revealed a single component at R_F 0.44 in chloroform–acetone–acetic acid (12 : 5 : 3) and a single peak by h.p.l.c., $[\alpha]_D^{20}$ −13 (*c* 1.0, EtOH) and −20° (*c* 1.0, 1% $NaHCO_3$), $\nu_{max.}$ (KBr) 3 300, 2 960, 2 950, 1 690, 1 640, 1 450, and 1 250 cm^{-1}, $\lambda_{max.}$ (EtOH) 221 nm (ε 11 200), δ_H [$(CD_3)_2SO$] 5.55 (1 H, m, 2-H), 2.05 (3 H, s, 15-CH_3), 1.05 (3 H, d, 14-CH_3), and 0.80 (3 H, d, 17-CH_3), δ_C [$(CD_3)_2SO$] 167.3 (C-1), 156.4 (C-3), 117.6 (C-2), 74.5 (C-5), 69.4 (C-13), 68.2 (C-7), 67.7 (C-6), 64.6 (C-16), 59.0 (C-11), 54.6 (C-10), 42.4 (C-4 and -12), 37.3 (C-8), 31.5 (C-9), 20.0 (C-14), 18.4 (C-15), and 11.6 p.p.m. (C-17), *m/e* 227 ($M^+ - H_2O - C_5H_7O_2$, 82%) 141 (43), and 111 (100) (Found: C, 59.0; H, 8.2. $C_{17}H_{28}O_7$ requires C, 59.3, H, 8.2%).

Sodium Monate A.—Monic acid A (3.44 g) was dissolved in water (10 ml). 0.1N-Sodium hydroxide solution (10 ml) was added until pH 7.5 was obtained. The solution was freeze dried and finally dried *in vacuo* over P_2O_5 to give *sodium monate A* (3.66 g), $[\alpha]_D^{20}$ −20° (*c* 1.0, H_2O), $\nu_{max.}$ (KBr) 3 400, 2 970, 1 650, and 1 550 cm^{-1}, $\lambda_{max.}$ (EtOH) 214 nm (ε 14 600), δ_H [$(CD_3)_2SO$] 5.16 (1 H, m, 2-H), 1.95 (3 H, s, 15-CH_3), 1.05 (3 H, d, 14-CH_3), and 0.79 (3 H, d, 17-CH_3), δ_C [$(CD_3)_2SO$] 172.4 (C-1), 141.2 (C-3), 127.5 (C-2), 75.0 (C-5), 69.3 (C-13), 68.5 (C-7), 67.7 (C-6), 64.5 (C-16), 59.1 (C-11), 54.6 (C-10), 42.6 (C-4 and -12), 37.9 (C-8), 31.6 (C-9), 20.1 (C-14), 17.6 (C-15), and 11.4 p.p.m. (C-17).

Methyl Monate A (3b) *from Monic Acid A* (3a).—Sodium monate (1.12 g) was dissolved in DMF (15 ml) with hexamethylphosphoramide (3 drops). Methyl iodide (5 ml) was added and the solution was stirred at room temperature for 18 h. The mixture was evaporated to dryness under re-

duced pressure and the oily residue partitioned between ethyl acetate and water. The ethyl acetate layer was separated, washed with sodium hydrogencarbonate solution and brine, dried ($MgSO_4$), and evaporated to an oil. The latter was dissolved in dry ether, from which methyl monate A (3b) crystallized as a solid (0.71 g, 65%), m.p. 124—125° [no depression of mixed m.p. with sample prepared from the ketone (2)], spectroscopically and chromatographically identical with previously prepared material.

Ethyl Monate A (3c) *from Monic Acid A* (3a).—Using an identical procedure to that described above sodium monate (0.80 g) afforded crystalline ethyl monate A (3c) (0.55 g, 68%), m.p. 99—100°, spectroscopically and chromatographically identical with material prepared from the ketone (2).

Methyl 9-Chlorononanoate.—A solution of 9-hydroxynonanoic acid (1.74 g) and thionyl chloride (2.2 ml) in dry benzene (50 ml) containing DMF (2 drops) was heated under reflux for 3.5 h. The mixture was cooled and the solvent removed under reduced pressure. The resulting 9-chlorononanoyl chloride was not isolated, but dissolved in anhydrous methanol (100 ml) and the solution heated under reflux for 18 h. Methanol was removed under reduced pressure and the resulting liquid dissolved in ether, washed with sodium hydrogencarbonate solution and brine, dried ($MgSO_4$), and evaporated to a liquid, which was distilled *in vacuo* to give pure methyl 9-chlorononanoate (1.99 g), b.p. 80—84° at 0.3 mmHg, n_D^{18} 1.392,[13] $\nu_{max.}$ (film) 2 950, 2 800, 1 810, and 730 cm^{-1}, δ_H 3.69 (3 H, s, CH_3O), 3.54 (2 H, t, $ClCH_2$), 2.35 (2 H, t, CH_2CO), and 1.8—1.0 (12 H, m, 6 × CH_2) (Found: C, 58.2; H, 9.5; Cl, 17.05. $C_{10}H_{19}ClO_2$ requires C, 58.1; H, 9.3; Cl, 17.15%).

Methyl Pseudomonate A (1b) *from Monic Acid A* (3a).—A solution of sodium monate (0.366 g), methyl 9-chlorononanoate (0.41 g), and sodium iodide (0.300 g) in DMF (5 ml) containing hexamethylphosphoramide (2 drops) was heated at 100° for 2.8 h. The mixture was cooled, evaporated to dryness, and the residue partitioned between ethyl acetate and sodium hydrogencarbonate solution. The ethyl acetate layer was washed with brine, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The resulting oily residue crystallized on trituration with ether–light petroleum (b.p. 40—60°) to give methyl pseudomonate A (0.299 g) m.p. 76.5—77° [chromatographically and spectroscopically identical with authentic (1b) with no depression of mixed m.p.].

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