Pseudomonic Acid. Part 1. The Structure of Pseudomonic Acid A, a Novel Antibiotic produced by *Pseudomonas fluorescens* †

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The structure (I) of pseudomonic acid A, 9-{4-[5-(2,3-epoxy-5-hydroxy-4-methylhexyl)-3,4-dihydroxytetrahydropyran-2-yl]-3-methylbut-2-enoyloxy}nonanoic acid, has been established from n.m.r., mass spectral, and degradative studies of its methyl ester and various derivatives.

A STRAIN of *Pseudomonas fluorescens* (NCIB 10586), when grown in submerged culture, produces a number of antimicrobially active, acidic substances,[1] which can be conveniently isolated and purified after esterification.[2,3] This paper deals with the structure elucidation of the methyl ester (IIa) of the major metabolite (I) which we have called pseudomonic acid A (pseudomonic acid [2,3]) that is responsible for a significant proportion of the antibacterial activity.

Methyl pseudomonate A, m.p. 77—78°, was shown to have the molecular formula $C_{27}H_{46}O_9$ on the basis of its composition and high resolution mass spectrum. Hence, the formula of the parent acid must be $C_{26}H_{44}O_9$. The methyl ester was optically active, $[\alpha]_D$ −9°. The u.v. and i.r. spectra [$\lambda_{max.}$ 221.5 nm (ε 13 400); $\nu_{max.}$ 1 715 and 1 650 cm^{-1}] indicated the presence of an αβ-unsaturated ester grouping. The i.r. spectrum, in addition to the methyl ester grouping ($\nu_{max.}$ 1 740 cm^{-1}), also indicated the presence of hydroxy-groups ($\nu_{max.}$ 3 600—3 100 cm^{-1}). The ^{1}H n.m.r. spectrum, in addition to the methoxy-singlet at τ 6.54, showed two doublets at τ 9.09 (3 H, *J* 7 Hz) and 8.81 (3 H, *J* 6.5 Hz) and a broad singlet at τ 7.84 (3 H), indicative of two secondary and one olefinic methyl groups. A broad singlet at τ 4.32 (1 H) was ascribed to an olefinic proton. The presence of *ca.* 10 methylene protons was also inferred from a broad singlet at τ 8.72. The proton-noise-decoupled ^{13}C n.m.r. spectrum ‡ of (11a) showed a total of 24 signals, three overlapping at 29.0 and two at 42.9 p.p.m. (to low field of Me_4Si), which were ascribed to the following groupings on the basis of chemical shift considerations and the multiplicities in the s.f.o.r.d. spectrum: 2 –COR, >C=CH–,

† Preliminary communication, ref. 3.

‡ Since the assignment of this spectrum was made after the structure (I) had been determined, full details have been included in the accompanying paper describing the biosynthesis of (I).[4]

[1] A. Baader and C. Garre, *Corresp.-Bl. Schweiz. Aerzte.*, 1887, **17**, 385.

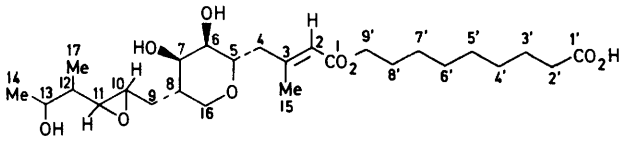
[2] A. T. Fuller, G. Mellows, M. Woolford, G. T. Banks, K. D. Barrow, and E. B. Chain, *Nature*, 1971, **234**, 416; Beecham Group Ltd., G.P. 2227 739.

[3] E. B. Chain and G. Mellows, *J.C.S. Chem. Comm.*, 1974, 847.

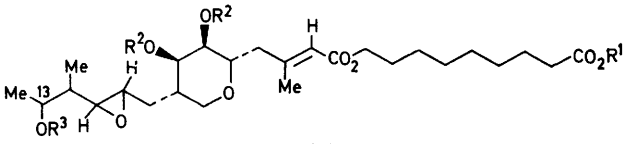
[4] T. C. Feline, R. B. Jones, G. Mellows, and L. Phillips, following paper.

6 >CH·O–, 2 –CH_2·O–, MeO–, 2 >CH–, 9 –CH_2–, and 3 Me–.

Methyl pseudomonate A formed a triacetate (IIb), $C_{33}H_{52}O_{12}$, and a tribenzoate (IIc), $C_{48}H_{58}O_{12}$, neither of which showed hydroxy absorbance in its i.r. spectrum. Comparison of the ^1H n.m.r. spectra of (IIa) and its



(I)



(II)

a; R^1=Me, R^2=R^3=H
b; R^1=Me, R^2=R^3=Ac
c; R^1=Me, R^2=R^3=Bz
d; R^1=CH_2·CO·C_6H_4 Br-*p*, R^2=R^3=H
e; R^1=Me, $(R^2)_2$=Me_2C, R^3=H
f; R^1=Me, $(R^2)_2$=Me_2C, R^3=CO·C_6H_4(N:N·C_6H_4·NO_2-*p*)-*p*
g; R^1=Me, $(R^2)_2$=Me_2C, R^3=Ac
h; R^1=R^2=R^3=Me

acylated derivatives showed that signals for three protons moved *ca.* 0.5—1 p.p.m. to lower field on acylation, indicating that pseudomonic acid A has three secondary alcohol functions. Similar observations were made for the *p*-bromophenacyl ester, (IId), $C_{34}H_{49}BrO_{10}$. The methyl ester (IIa) also formed an acetonide (IIe), $C_{30}H_{50}O_9$, which formed a crystalline 4-(*p*-nitrophenylazo)benzoate (IIf), $C_{43}H_{57}N_3O_{12}$, m.p. 72—74°, indicating that two of the secondary hydroxy-groups formed an α-glycol system. This was further substantiated by the formation of an unstable dialdehyde (III), $\nu_{max.}$ 2 710, 1 740, 1 715, and 1 650 cm^{-1}, τ 0.36 (2 H, m, CHO), on treatment of (IIa) with sodium periodate (1 mol. equiv. uptake). Also, spin–spin decoupling from the α-glycol proton at τ 5.31 in the ^1H n.m.r. spectrum of the triacetate (IIb), resulted in the loss of the 3 Hz coupling to the proton resonating at τ 4.78 [sharpened to a doublet (*J* 3 Hz)]. The lower field secondary methyl group [τ 8.77 (d, *J* 7 Hz)] was also shown to be coupled to the remaining secondary acetate proton (τ 5.07). Further decoupling experiments on these derivatives were not possible at 100 MHz because of extensive overlap of signals in the other parts of the spectra. Seven of the nine oxygen atoms in (IIa) were now accounted for. Of the remaining two, it was inferred from the chemical shift [5a] of the two incompletely resolved signals at τ 7.45—7.20 in (IIa), which were more clearly discerned as a double doublet at τ 7.38 (*J* 2 and 7.5 Hz) and a finely split triplet at τ 7.21 (*J* 2 and 6 Hz) in the spectrum of the acetonide (IIe), that one was present as an epoxide grouping. This was proved by evidence presented later. At this point, with no evidence of unsaturation other than that provided by the αβ-unsaturated ester grouping, the remaining oxygen must therefore occupy an ethereal linkage, which must also be present in a ring.

Methyl pseudomonate A (IIa) and its triacetate (IIb) both absorbed 1 mol. equiv. of hydrogen on catalytic hydrogenation over platinum to give the respective dihydro-derivatives, (IVa), $C_{27}H_{48}O_9$, and (IVb), $C_{33}H_{54}O_{12}$, which showed only end absorbance in their u.v. spectra. In the formation of (IVa), the ^1H n.m.r. spectrum showed the loss of the olefinic proton and methyl group in (IIa) and the appearance of a new doublet signal at τ 9.08 (3 H, *J* 7 Hz). The i.r. spectrum of (IVa) also showed the loss of the αβ-unsaturated ester bands at 1 715 and 1 655 cm^{-1}, and the methyl ester carbonyl band at 1 740 cm^{-1} increased in intensity. These observations confirm the trisubstituted nature of the αβ-unsaturated ester grouping in (IIa) to which the olefinic methyl group is attached.

Treatment of methyl pseudomonate A (IIa) with methanolic potassium hydroxide yielded 9-hydroxynonanoic acid (Va), which formed a crystalline *p*-bromophenacyl ester (Vb), $C_{17}H_{23}BrO_4$, m.p. 77.5—78°, characterised by its mass spectral fragmentation pattern. Treatment of the *p*-bromophenacyl ester (IId) with Lemieux–Rudloff reagent [6] [potassium permanganate and sodium periodate in t-butyl alcohol] also afforded (Vb). Further conformation of the 9-hydroxynonanoate moiety in (I) came from the isolation of 1,9-dihydroxynonane (VI), m.p. 46° (bisphenylcarbamate derivative, m.p. 168—169°), whose m.p.s agreed well with literature citations [7] (42.5—44.5 and 171.5—172.5°, respectively), from reduction of (IVa) with lithium aluminium hydride. The mass spectra of (IIa) and its derivatives (see later) also provided evidence for the C_9 unit. Thus, (I) contains the biogenetically unusual 9-hydroxynonanoic acid moiety, the hydroxy-group of which is attached to the rest of the molecule through an αβ-unsaturated ester linkage.

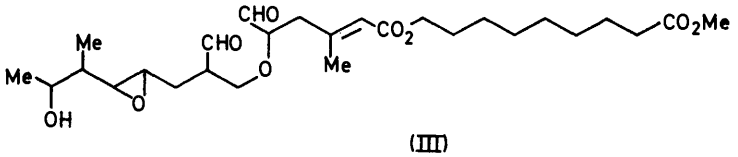
In order to provide a suitable derivative for extensive ^1H n.m.r. spin–spin decoupling experiments, attempts were made to remove cleanly the C_9 unit. In the basic hydrolysis reaction two acidic products in addition to (Va) were formed. After separation as their methyl esters (after treatment with the diazomethane) on p.l.c., the two products were benzoylated, affording tribenzoates whose n.m.r. spectra lacked the methylene envelope indicating that the αβ-unsaturated ester unit had been hydrolysed. However, the two epoxide protons were missing in both spectra indicating that the epoxide

[5] (*a*) L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' 2nd edn., Pergamon, London, 1969, p. 171; (*b*) J. W. K. Burrell, L. M. Jackman and B. C. L. Weedon, *Proc. Chem. Soc.*, 1959, 263; (*c*) L. M. Jackman and R. H. Wiley, *J. Chem. Soc.*, 1960, 2886; (*d*) L. Jackman, R. Ruegg, G. Ryser, C. van Planta, U. Gloor, H. Mayer, P. Schudel, M. Kofler, and O. Igler, *Helv. Chim. Acta*, 1965, **48**, 1332; (*e*) R. Azarad and M.-O. Cyrot, *Bull. Soc. chim. France*, 1965, 3740.

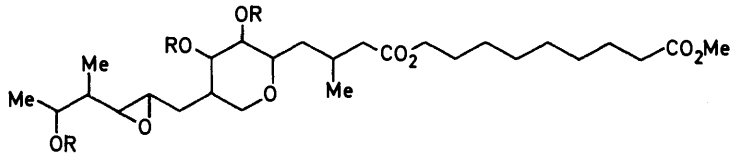
[6] L. F. Fieser and M. Fieser, 'Reagents for Organic Synthesis,' Wiley, New York, 1967, p. 810.

[7] S. G. Polyakova and V. N. Belov, *Zhur. obshchei Khim.*, 1964, **34**, 565.

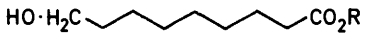
grouping had been modified. Also the olefinic proton in the least polar compound had disappeared. These two substances remain uncharacterised. In the reduction of



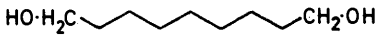
(III)



(IV)
a; R = H
b; R = Ac



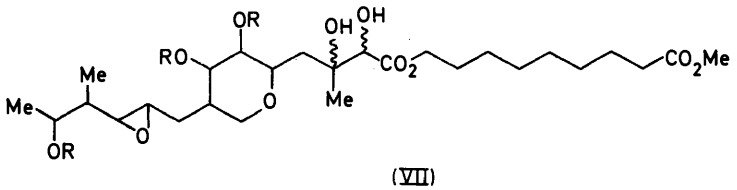
(V)
a; R = H
b; R = $CH_2 \cdot CO \cdot C_6H_4Br$-$p$
c; R = Me



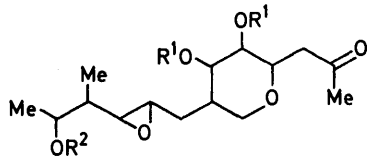
(VI)

(IVa) by lithium aluminium hydride at least four inseparable alcohols lacking the C_9 unit were formed, as evidenced from the n.m.r. spectrum of the mixture, in

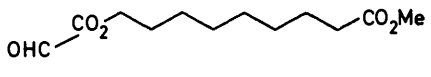
of the tetra-acetate (IIb) with osmium tetraoxide in pyridine followed by work-up with disulphite generated a mixture of diastereoisomeric diols (VIIa), $\nu_{max.}$ 3 600—3 200 cm^{-1} (OH). The diols were not further characterised and were cleaved in good yield to a mixture of the oily methyl ketone triacetate (VIIIa), $C_{21}H_{32}O_9$, and the



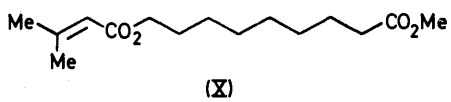
(VII)
a; R = Ac
b; R = Bz



(VIII)
a; $R^1 = R^2$ = Ac
b; $R^1 = R^2$ = Bz
c; $(R^1)_2 = Me_2C$, R^2 = Ac



(IX)



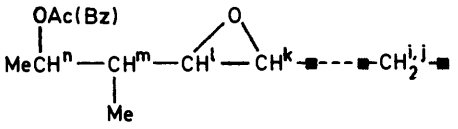
(X)

addition to (VI). Initial attempts at ozonolysis of (IIa) followed by reductive work-up with zinc and acetic acid also generated multi-component mixtures. The C_9 side chain was successfully removed as follows. Treatment

oily 8-methoxycarbonyloctyl glyoxylate (IX), $C_{12}H_{20}O_5$, characterised as its semicarbazone, $C_{13}H_{23}N_3O_5$, m.p. 164—165.5°. The methyl ketone tribenzoate (VIIIb), $C_{36}H_{38}O_9$, and methyl ketone acetonide acetate (VIIIc),

$C_{20}H_{32}O_7$, were obtained similarly. The ^1H n.m.r. spectra of compounds (VIIIa—c) showed, in addition to the two secondary methyl signals, a singlet methyl signal [*e.g.* τ 7.86 for (VIIIa)], whose chemical shift indicated attachment to a ketone function and which could only have arisen from the olefinic methyl group of (IIb, c, or g). This established that the olefinic methyl group must reside on the β-carbon atom of the αβ-unsaturated ester grouping in (I). Furthermore, the chemical shift of the olefinic methyl group [τ 7.84 for (IIa)] is in excellent agreement with those reported for β-methyl-substituted *trans*-(*E*-)αβ-unsaturated esters.[5,*] [The chemical shift of the β-methyl group in β-methyl substituted *cis*-(*Z*-)αβ-unsaturated esters occurs *ca.* 0.2—0.3 p.p.m. to higher field owing to the shielding influence of the ester function.[5]] The same conclusion was also reached from a comparison of ^{13}C n.m.r. chemical shift of the vinylic methyl group in (IIa) [19.1 p.p.m. to low field of Me_4Si] with those of the two vinylic methyl groups of 8-methoxycarbonyloctyl 3-methylbut-2-enoate (X) (*cis*-Me 20.1 p.p.m.; *trans*-Me 27.4 p.p.m.) and with literature citations [9] (see accompanying paper [4] for details).

Extensive spin–spin decoupling experiments on the methyl ketone derivatives (VIIIa—c) allowed the formulation of two part structures. The ^1H chemical shifts of the two secondary methyl groups (τ 8.75 and 9.04) showed no change on conversion of (IIb) into (VIIIa), implying that they were remote from the newly created methyl ketone group. The lower field doublet at τ 8.75 (*J* 6.5 Hz) was assigned to the end-of-chain methyl group and was coupled to the non-glycolic secondary alcoholic proton (H^n), which gave rise to a quintet at τ 5.06 (*J* 7 Hz). This was in turn coupled to a proton resonating at τ 8.50 (H^m) as a multiplet. Decoupling from the higher field secondary methyl doublet at τ 9.04 sharpened this to a double doublet (*J* 7 and 8 Hz). Decoupling from the τ 8.50 proton simultaneously caused collapse of the methyl doublet (τ 9.04) to a singlet and removed the 8 Hz coupling from the higher field epoxide proton signal (H^l) at τ 7.31, which now appeared as two lines (*J* 2.2 Hz). Because of the nearly identical chemical shifts of the two epoxide protons (τ 7.31 and 7.35) it was not possible to continue the sequential decoupling by direct irradiation experiments. This was overcome by applying the homo-INDOR technique to the acetonide acetate (IIg). The two protons (H^l and H^k) resonating at τ 7.39 and 7.27 in (IIg) have greater chemical shift difference, and that they were vicinally coupled to each other (*J* 2.2 Hz) was clearly demonstrated. The magnitude of this coupling is in good agreement with couplings reported [5] for *transoid* orientations of disubstituted epoxides; *cisoid* vicinal couplings being *ca.* 2 Hz greater. The triplet nature of signal due to the lower field epoxide proton (H^k) suggests that it is coupled to the protons of a methylene group. Irradiation at the frequency corresponding to H^k [τ 7.35 in (VIIIa) and 7.19 in (VIIIb)] caused substantial changes in the shape of a two-proton multiplet at τ 8.20 or 8.06, respectively. Reverse irradiation in both derivatives altered the shape of the H^k signal but the resulting multiplicity could not be clearly discerned. These decoupling experiments support part structure (A), and strongly suggest that the epoxide group is further bonded to a methylene group, the chemical shift of whose protons indicates that it is not bonded to oxygen.



(A)

The two protons (H^a and H^b) resonating at τ 7.60 and 7.40 in the spectrum of (VIIIa) [τ 7.48 and 7.28 in that of (VIIIb)] both appeared as double doublets (*J* 16 and 5 and *J* 16 and 8 Hz, respectively). The larger coupling indicated that they were mutually coupled, forming the AB part of an ABX system. The X part (H^c) of the spectrum was located at τ 5.84 as an octet, and was further coupled (*J* 10 Hz) to the α-glycolic proton (H^d) resonating at τ 5.28 as a double doublet. The other coupling (*J* 3 Hz) involved the remaining α-glycolic proton (H^e), which resonated at τ 4.76 as a triplet (*J* 3 Hz), indicating that it was bonded to a methine carbon atom ($J_{e,f}$ 3 Hz). The latter (H^f), formed the X part of a second ABX system and resonated at higher field, its signal being obscured by the acetate methyl signals, at τ 8.02. Decoupling at this position clearly sharpened the signal at τ 4.76 to a doublet, having lost one of the 3 Hz couplings, and also removed the smaller couplings (*J* 3 and 1.5 Hz) of the two protons (H^g and H^h) at τ 6.10 and 6.38, which formed the AB part of the second ABX system; H^g and H^h were also mutually coupled (*J* 12 Hz). The chemical shifts of these two protons indicate that this methylene group must be bonded to an oxygen atom, which must be the ethereal oxygen present in the ring. Since all the oxygen atoms in (VIIIa) were now accounted for, the ethereal oxygen must form a six-membered ring by bridging the aforementioned methylene group and the carbon atom bearing H^c (τ 5.84). Although the H^f resonance was hidden under the acetate signals in the spectrum of (VIIIa), it was partly visible in the spectrum of (VIIIb) and clearly changed in shape on decoupling from either H^g and H^h. At this point a second part structure (B) can be formulated.

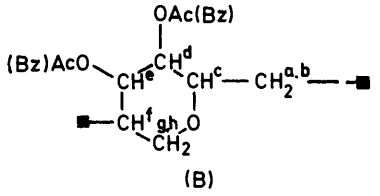
The chemical shifts of H^a and H^b in (VIIIa—c) [*e.g.* 7.60 and 7.40 in (VIIIa)] and the enhanced geminal coupling ($J_{a,b}$ 16 Hz), suggest that the methyl ketone moiety is attached at this point. This is strongly supported by the mass spectral fragmentation patterns of (IIa) and its derivatives and by biosynthetic evidence.[4]

* The *E*-configuration has been further substantiated by a study of the nuclear Overhauser enhancements seen in the ^1H n.m.r. spectra of *E*- and *Z*-isomers of (IIa).[8]

[8] R. G. Alexander, J. P. Clayton, K. Luk, and N. H. Rogers, in preparation.

[9] J. B. Stothers, 'C-13 N.m.r. Spectroscopy,' Academic Press, London, 1972, pp. 80—85 and 183—195 and references cited.

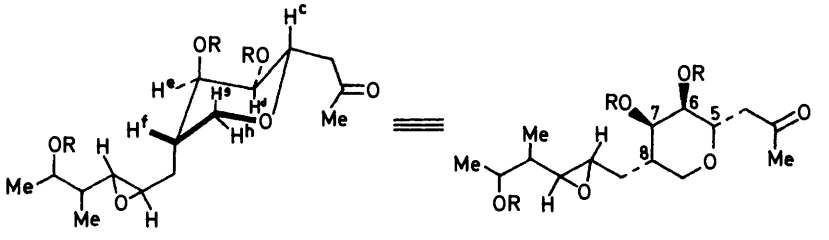
This leaves only one methylene group unaccounted for, the signal of which can be partly discerned in the n.m.r. spectra of all the methyl ketone derivatives (VIIIa—c)



(B)

and (XIV). The only way this methylene group can be accommodated in the complete structure of (VIIIa and b) is by joining the two part structures (A) and (B) through the epoxide carbon atom of the former and the methine carbon atom of the latter.

As shown by Dreiding models, the values of the coupling constants of the pyran ring protons in (VIIIa and b) were consistent with the ring adopting a *Cl* conformation. The relatively large J_{vic} value (10 Hz) between H^c and H^d disclosed the antiperiplanarity of these protons; hence the methyl ketone side chain and the C-5 acyloxy-group must be equatorially disposed. The small vicinal couplings between the ring methyleneoxy protons (H^g and H^h) and H^f (1.5 and 3 Hz, respectively) pointed to an axial orientation for the epoxide side chain, leaving H^f equatorially disposed. Of the two C-16 protons (H^g and H^h), the axial proton (H^g) was assigned to the higher field doublet at τ 6.38 in (VIIIa), with the smallest J_{vic} value, because of the antiperiplanar orientation of the ring oxygen with H^f [10] [heavy line in (XI)]. The small values of the vicinal couplings $J_{d,e}$ and $J_{e,f}$ (3 Hz each) are in accord with *ax,eq* and *eq,eq* relationships for H^d and H^e and H^f, respectively; hence the C-6 acyloxy-group is axially disposed. The relative stereochemistry at C-5, -6, -7, and -8 must therefore be as depicted in (XI).

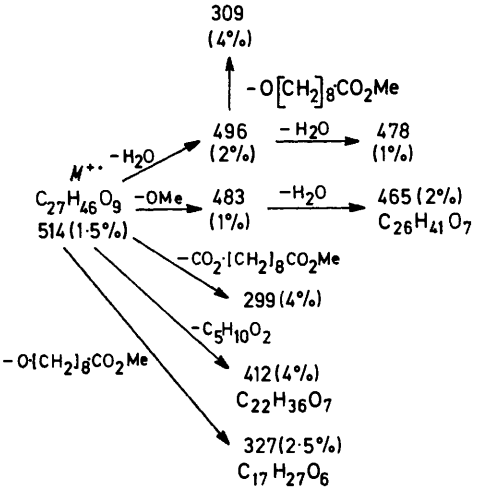


(XI)

Hence, the complete structure of methyl pseudomonate A must be (IIa) and that of pseudomonic acid A (I), with the relative stereochemistry of the pyran ring as shown. The same conclusion has also been reached independently from a detailed study of the ^1H n.m.r. spectrum of (IIa) at 300 MHz.[11]

The mass spectra of methyl pseudomonate A and its derivatives were particularly informative. In the mass spectrum of (IIa) the majority of the peaks of significant intensity (>20%) appeared at *m/e* values below that of base peak at *m/e* 111. With the exception of the peaks at *m/e* 227 and 270 the fragments above *m/e* 111 were of less than 9% relative intensity, and most of these can be accounted for by the fragmentation paths shown in Schemes 1—8. The formulae indicated for the fragments were unambiguously obtained from accurate mass measurements, and the Schemes are supported by metastable peaks (*m**) where indicated.

A major fragmentation path of (IIa) (Scheme 2) involves initial ionisation of the six membered ring oxygen followed by cleavage of the C(4)–C(5) bond to give the ion (XII), which readily loses two molecules of water to give the ions at *m/e* 227 (65%) and 209 (16%). This fragmentation, and the formation of the ions at *m/e* 83, 82, and 55 (Scheme 4), indicates the attachment of a methyl-ene group to the ketone moiety of the methyl ketones (VIIIa—c) and (XIV). Alternatively, (XII) may undergo hydrogen transfer to the epoxide oxygen, generating

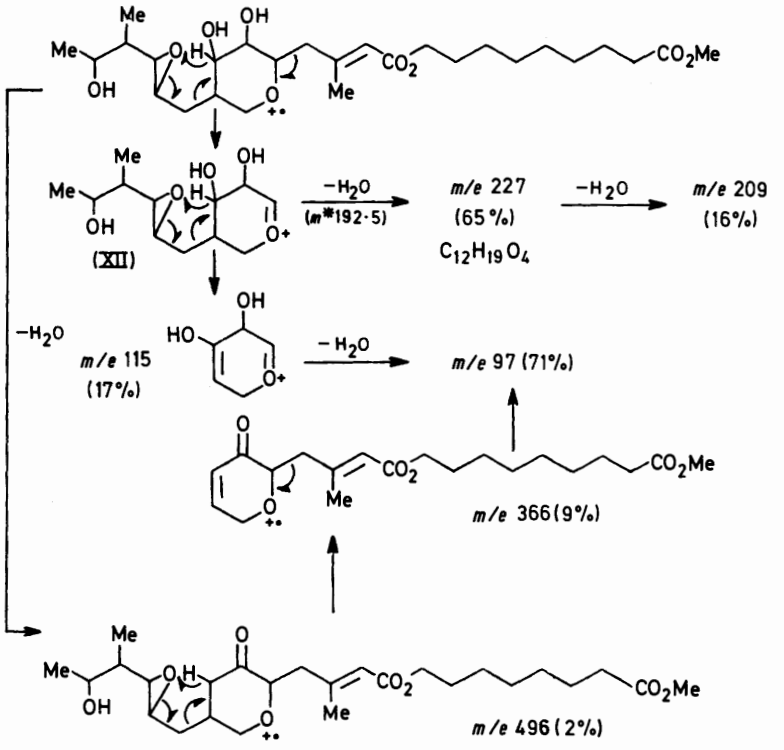


SCHEME 1

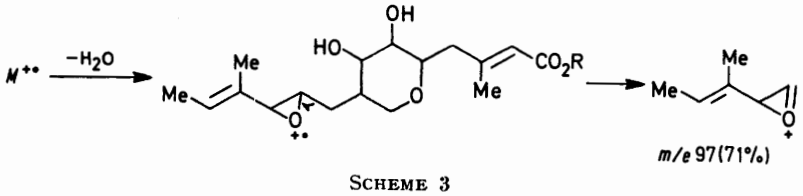
the ion *m/e* 115 (17%) which loses water to give *m/e* 97 (71%). The largest mass fragment, *m/e* 366 (9%), in the higher region of the spectrum could result from

[10] S. Sternhell, *Quart. Rev.*, 1969, 251; H. Booth, *Tetrahedron Letters*, 1965, 411.

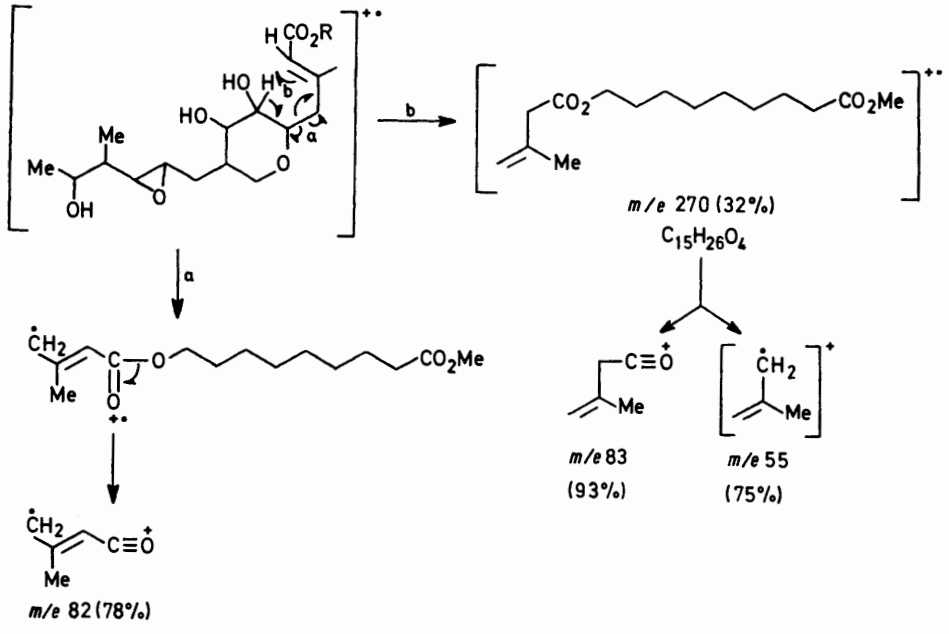
[11] M. Anteunis, A. De Bruyn, G. Mellows, and G. Verhegge, in preparation.



SCHEME 2

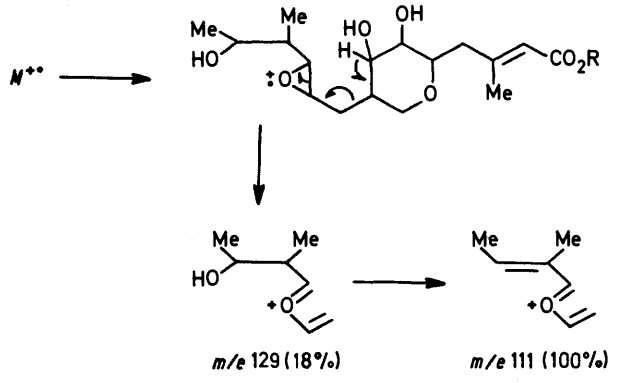


SCHEME 3



SCHEME 4

a similar hydrogen transfer to the epoxide following loss of water from the molecular ion, which could also fragment to *m/e* 97. A further contribution to



SCHEME 5

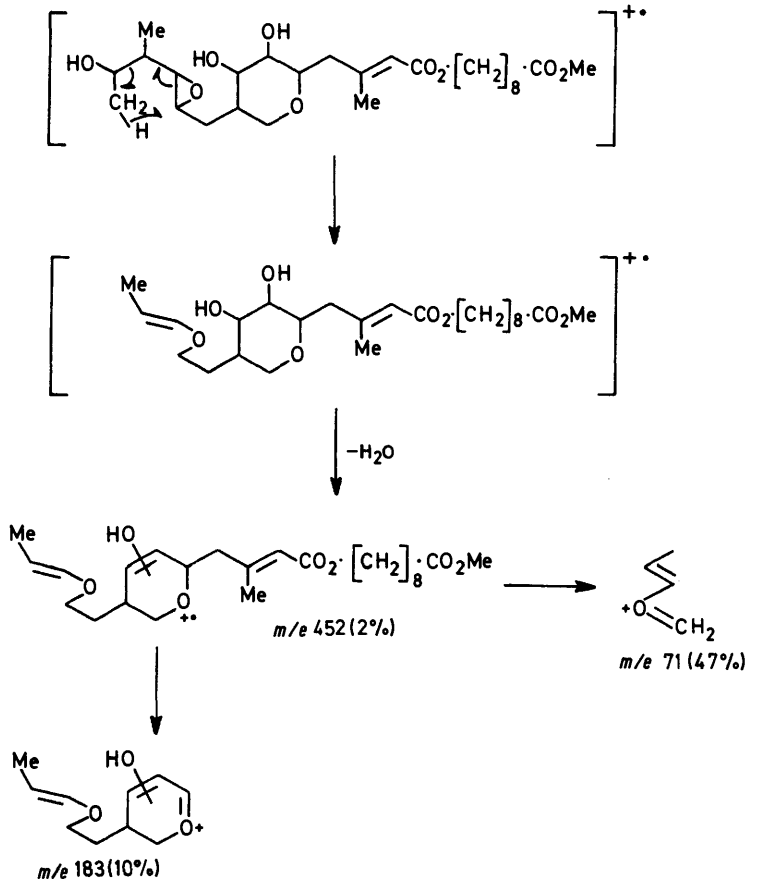
m/e 97 would arise from initial ionisation of the epoxide oxygen in (IIa) followed by cleavage of the adjacent bond on the pyran ring side and loss of water (Scheme 3) [the same ion was also seen in the spectra of (IIb) and (IVb)].

A second major fragmentation pathway (Scheme 4) involves hydrogen transfer to the double bond with subsequent cleavage of the αβ-unsaturated ester grouping from the pyran ring to give *m/e* 270 (32%), which would be expected to furnish the ions *m/e* 83 (95%) and 55 (75%) on further fragmentation. The ion *m/e* 82 (78%) is explained by cleavage of the C(4)–C(5) bond of M^+ followed by loss of the 9-hydroxynonanoate moiety. The latter can also be lost directly from $M^{+\cdot}$ (Scheme 1) yielding *m/e* 327 (2.5%), $C_{17}H_{27}O_6$, which in turn loses water to give *m/e* 309 (4%).

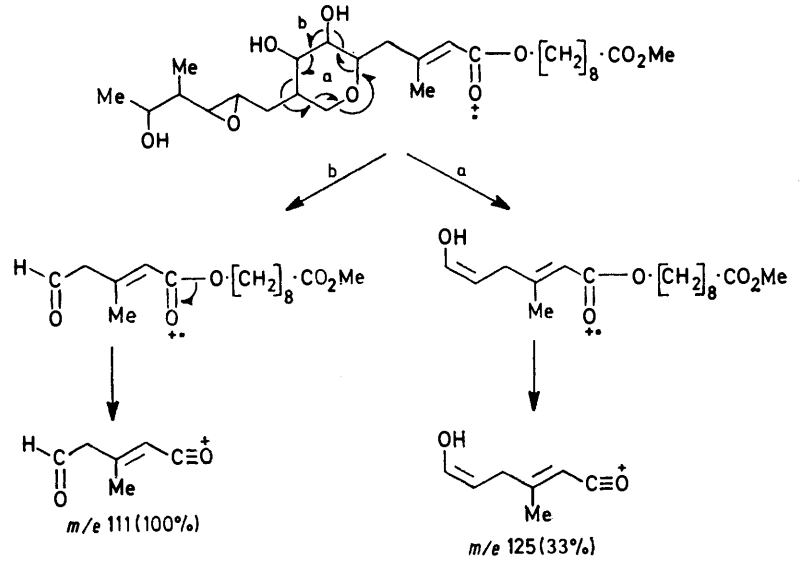
The base peak at *m/e* 111, which appeared in the spectrum of (IIb) (89%), is probably formed by loss of water from the ion *m/e* 129 (8%), which would arise by loss of hydrogen from one of the α-glycol carbon atoms following initial ionisation of the epoxide oxygen (Scheme 5).

The assignment of the remaining ions in the spectrum is more speculative. However, the significant ions at *m/e* 452 (2%), 183 (10%), and 71 (47%) and at *m/e* 125 (33%) could arise by the fragmentation paths shown in Schemes 6 and 7.

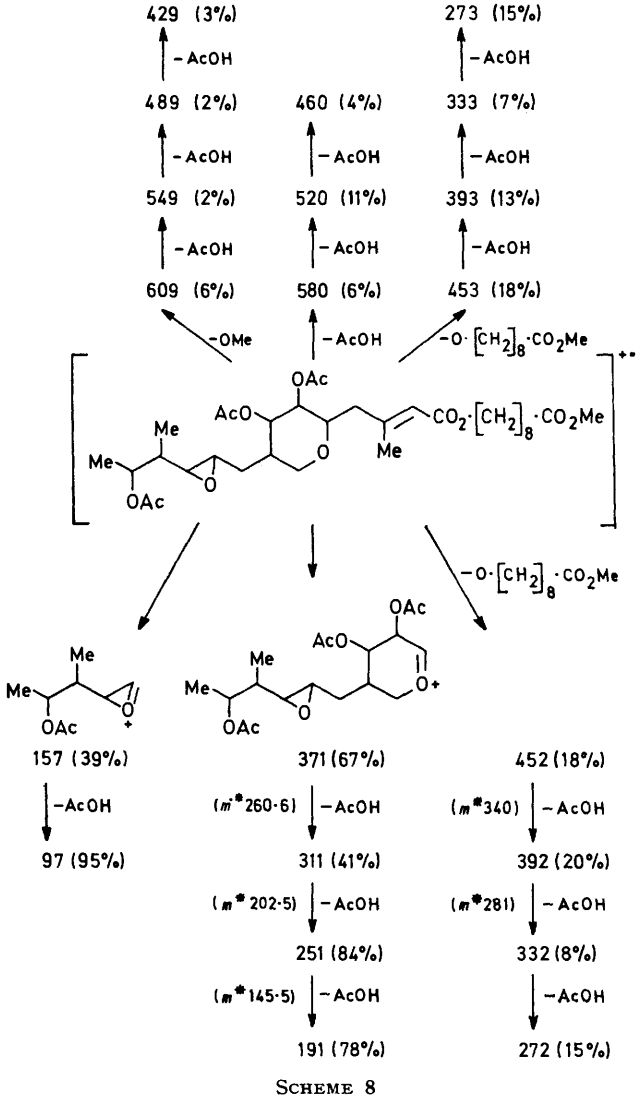
The mass spectrum of the triacetate (IIb) had many features in common with that of (IIa), but the lower mass end of the spectrum was difficult to interpret, showing a continuous series of equally abundant peaks down to the acetyl fragment base peak at *m/e* 43. In the higher mass region, several fragmentation paths, involving the sequential loss of three acetic acid residues, occurred



SCHEME 6



SCHEME 7



SCHEME 8

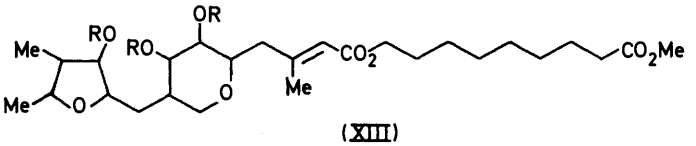
(Scheme 8). The major fragmentation route again involved cleavage of the C(4)–C(5) bond generating *m/e* 371 (67%), which sequentially lost three molecules of acetic acid. In the spectrum of the dihydro-triacetate (IVb), whose highest mass peak occurred at *m/e* 611 (4%) ($M^{+\cdot}$ — ·OMe) and was generally uninformative, this fragmentation route was absent.

With regard to the structure of pseudomonic acid A, these fragmentation Schemes add weight to the location of the epoxide group in the side chain and the attachment of a methylene group as well as the methyl group to the β-carbon atom of the double bond, and provide additional evidence for the nature and attachment of the 9-hydroxynonanoic acid moiety.

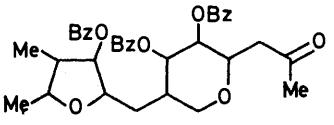
In the early part of this investigation, several abortive attempts were made to identify chemically the epoxy-group. The failure of the reduction of (IVa) with lithium aluminium hydride to yield identifiable products in addition to (VI) has already been mentioned. Treatment of (IIa) and (IVa) under a variety of acidic conditions (*e.g.* aqueous 3% perchloric acid in tetrahydrofuran; periodic acid in ether [12]) in an attempt to open the epoxide to give the corresponding diol also generated inseparable mixtures, presumably arising through cyclisation of the resulting diol with one or more of the other hydroxy-groups. To prevent further condensations of this type, the tribenzoate (IIc) was treated with 60% perchloric acid in ethyl acetate [13] (other conditions gave poorer yields). A ready rearrangement took place to give a crystalline, slightly less polar, isomeric compound, $C_{36}H_{48}O_{12}$, which was assigned structure (XIIIa) on the following evidence. Comparison of the ^{1}H n.m.r. spectra of (IIc) and (XIIIa) indicated that the substituted perhydropyran ring and the αβ-unsaturated ester unit had remained intact. The product also contained three

[12] Ref. 6, p. 817.
[13] J. M. Diggle, M. D. Halliday, A. Kasal, G. D. Meakins, and M. S. Saltmarch, *J. Chem. Soc.* (*C*), 1970, 2325.

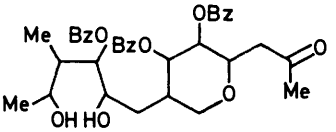
benzoate groups. The signals of the two epoxide protons (H^k and H^l) had shifted downfield from their normal position at τ 7.07 and 7.19 in (IIc) to the ether proton region, τ 5.9—6.6. Also, the non-glycolic secondary benzoate proton signal had shifted upfield into the same region and the chemical shifts of the two secondary methyl signals had changed. To allow spin–spin decoupling experiments, the side chain was removed from (XIIIa) by the osmium tetraoxide method to give the crystalline methyl ketone tribenzoate (XIV), $C_{36}H_{38}O_9$, m.p. 138.5—140°, and (IX). Comparison of the ^1H n.m.r. spectra of (VIIIb) and (XIV) clearly showed that chemical shifts and spin multiplicities of the protons in the pyran ring were essentially unchanged. Decoupling from the lower field secondary methyl located the H^n signal at τ 6.53, shifted upfield by 1.73 p.p.m. from its position in (VIIIb). Under irradiation conditions it appeared as a doublet, J 7 Hz, having lost the 6.5 Hz coupling from the methyl group. This proton was in turn coupled to H^m (τ 8.12), geminal to the other secondary methyl group. Decoupling from H^m simultaneously caused collapse of the methyl doublet to a singlet and removed the 3 Hz coupling from H^l which resonated as a double doublet (J 3 and 5 Hz) at τ 4.95. The carbon atom to which H^l, previously the lower field epoxide proton, is attached must therefore carry the rearranged benzoate group. Further irradiation at the H^l frequency located the H^k signal at τ 5.94, which sharpened from a poorly defined double doublet (J 5 and 6 Hz) to a triplet (J 6 Hz), having lost the 5 Hz coupling. The chemical shift of H^k in (XIV) suggests that it is now in an ether linkage. Although the spin multiplicities of H^i and H^j, to which H^k is coupled, could not be unambiguously discerned in either 100 or 220 MHz spectra, the signals were clearly located at τ 7.9—8.1 in the 220 MHz spectrum of (XIV). The triacetate (IIb) also underwent the same rearrangement (but more slowly) to a triacetate, $C_{33}H_{52}O_{12}$, formulated as (XIIIb) on the basis of its ^1H n.m.r. and mass spectra.



(XIII)
a; R = Bz
b; R = Ac

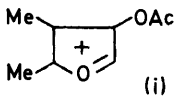


(XIV)



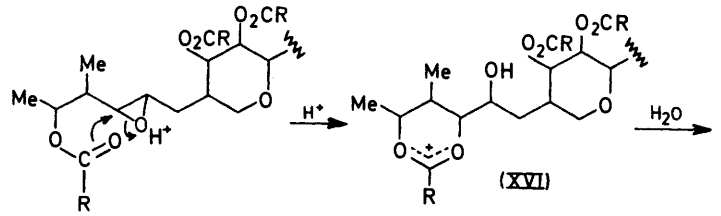
(XV)

The mass spectrum of (XIIIb), $M^{+\cdot}$ 640, had many features in common with that of (IIb). In particular the formation of the ion at m/e 371 (10%) with subsequent loss of three molecules of acetic acid to give m/e 191 (6%), supports the intact nature of the six-membered ring and its $\alpha\beta$-unsaturated ester attachment. The formation of the ion at m/e 157 (13%) ascribed to (i), which readily

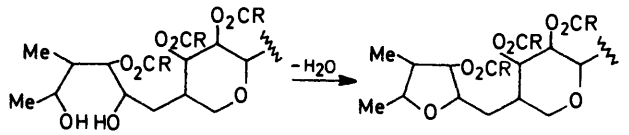


(i)

loses acetic acid to give m/e 97 (25%), provided further evidence for the substituted perhydrofuran ring in (XIIIa and b).



(XVI)

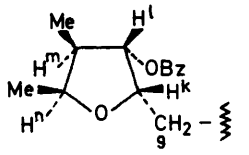


(XVII)

In the acid-induced rearrangement of (IIc), a small amount of a more polar compound, tentatively identified as (XV) from its n.m.r. spectrum, was isolated after con-

version into the methyl ketone derivative. When resubjected to the reaction conditions, (XV) was converted into (XIV) in high yield (t.l.c.). This rearrangement therefore presumably takes place by the prior formation of the acyloxonium ion (XVI), which suffers hydrolysis to the diol (XVII), subsequent dehydration of which furnishes the tetrahydrofuran derivatives.

The coupling constants for the protons in the furan ring suggest the relative stereochemistry depicted in (XVIII), in which the two methyl groups are *cis*-oriented, lying on the opposite face of the ring to the

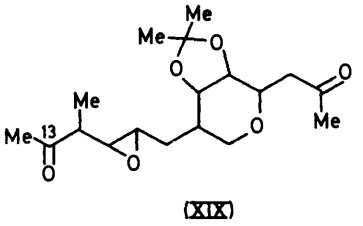


$J_{m,n}$ 6 Hz
$J_{l,m}$ 3 Hz
$J_{k,l}$ 4·5 Hz

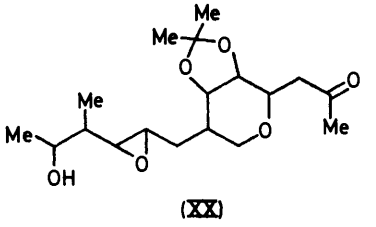
(XVIII)

benzoate and C-9 substituent.[14] It has not been possible to relate the relative stereochemistry of the four asymmetric centres of the epoxide side chain in (IIc), which one of the ketone groups had moved into conjugation [$\nu_{max.}$ 3 600 (OH) and 1 720 and 1 675 cm^{-1} ($\alpha\beta$-unsaturated ketone)]. This displacement could only be explained by the presence of an epoxide group β to the C-13 ketone group.

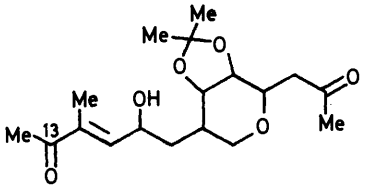
To prevent the acyl rearrangement described above (IIa) was converted into its trimethyl ether (IIh), $C_{30}H_{52}O_9$, $\lambda_{max.}$ 220.5 nm (ε 7 250), τ 6.67, 6.60, and 6.52 (3 OMe), with methyl iodide and sodium hydride in dimethylacetamide. The trimethyl ether (IIh) was cleaved to the two aldehydes (XXII) and (XXIII) with anhydrous periodic acid in ether,[12] and these were converted, *in situ*, into their respective 2,4-dinitrophenylhydrazones. The two derivatives were separated by p.l.c. The least polar compound was obtained in crystalline form, m.p. 76—78°, and identified as the 2,4-dinitrophenylhydrazone of 2-methyl-3-methoxybutyraldehyde (XXIIb), $C_{12}H_{16}N_4O_5$, which was optically active, $[\alpha]_D$ +6°. The more polar oily compound, $C_{30}H_{44}N_4O_{11}$, gave spectroscopic data in agreement with structure (XXIIIb) and was also optically active. These observations confirmed the presence of the epoxy-group in (I).



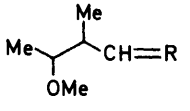
(XIX)



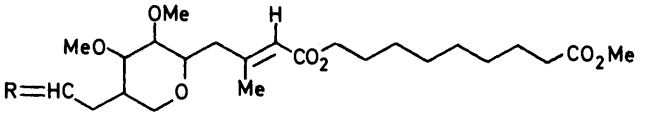
(XX)



(XXI)



(XXII)



(XXIII)

a ; R=O

b ; R=N·NH·C_6H_3–$(NO_2)_2$-2,4

because of the uncertainty of the nature and number of inversions of configuration which occur during the conversion (IIc) ⟶ (XIIIa).

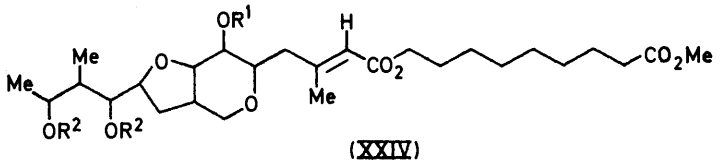
Treatment of the acetonide diketone (XIX), formed from (XX) by oxidation with chromium trioxide in pyridine, with potassium t-butoxide in t-butyl alcohol under conditions in which (XX) did not react, generated an unstable hydroxy-diketone, presumably (XXI), in

We have previously noted that pseudomonic acid A is unstable outside the range pH 4—9, as measured by bioassay of antibiotic activity.[2] On one occasion, during the work-up of a large-scale fermentation extract, the pH was unintentionally allowed to remain below 4 for 48 h. After the usual isolation and purification

[14] D. Gagnaire and P. Vottero, *Bull. Soc. chim. France*, 1963, 2779.

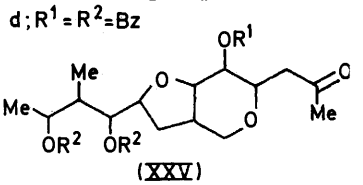
procedures, following methylation with diazomethane, an oily compound presumably derived from (I) and having the same chromatographic mobility as (IIa) was obtained. The new compound, $\lambda_{max.}$ 223 nm (ε 11 300), $[\alpha]_D$ −9°, was isomeric with (IIa) and was tentatively assigned structure (XXIVa) on the following evidence. The new compound did not react with periodate, indicating that the α-glycol system was no longer present. In its proton-noise-decoupled ^{13}C n.m.r. spectrum, (XXIVa) showed 24 signals; the resonances of C-1′—9′, C-1—4, C-8, C-9, C-12, and C-14—17 were identified by comparison of their chemical shifts and multiplicities in the off-resonance spectrum with those of (IIa).[4] No attempt was made to assign the >CH·O− signals. However, it was clear that the epoxide carbon signals (C-10 and C-11), which occurred to high field of the remaining >CH·O− signals in (IIa), had moved downfield, suggesting that the epoxide group had been modified. On treatment with 2,2-dimethoxypropane and toluene-*p*-sulphonic acid, (XXIVa) formed an acetonide (XXIVb), $C_{30}H_{48}O_9$, $\nu_{max.}$ 3 590 (OH) cm^{-1}, $\lambda_{max.}$ 222.5 nm (ε 11 200), which readily formed a monobenzoate (XXIVc), $\lambda_{max.}$ 222.5 nm (ε 12 300), $\nu_{max.}$ 1 740, 1 270, and 1 150 cm^{-1}. The latter was converted into the methyl ketone derivative (XXVa), $C_{25}H_{34}O_7$, by the osmium tetraoxide–sodium periodate procedure.

The ^1H n.m.r. spectrum of (XXVa) showed signals for H^k and H^l further downfield than in the epoxy-derivatives, in the region (τ 6.10—6.85) for protons bonded to ether carbon atoms. The signals for the two geminal protons (H^a and H^b) were clearly identified as double doublets from their chemical shifts (τ 7.42 and 7.08, respectively) and the enhanced geminal coupling ($J_{a,b}$ 16 Hz). Each was coupled to H^c (τ 5.41), which gave an eight-line signal ($J_{a,c}$ 6, $J_{b,c}$ 10 Hz). The remaining coupling ($J_{c,d}$ 1 Hz) involved H^d, which resonated at τ 4.85 as a poorly resolved double doublet. Decoupling from the τ 5.41 signal sharpened the latter signal into a doublet ($J_{d,e}$ 2.5 Hz). The H^e resonance was located at τ 6.72 as a double doublet, which collapsed to a doublet ($J_{e,f}$ 10 Hz) on irradiation at the H^d frequency. The chemical shift of H^e indicated that it was now bonded to an ether carbon atom. Further decoupling experiments were precluded by the extensive overlap of the remaining signals.



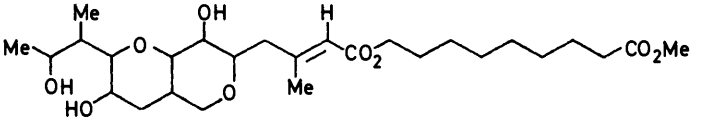
(XXIV)

a; $R^1 = R^2 = H$
b; $R^1 = H, (R^2)_2 = Me_2C$
c; $R^1 = Bz, (R^2)_2 = Me_2C$
d; $R^1 = R^2 = Bz$

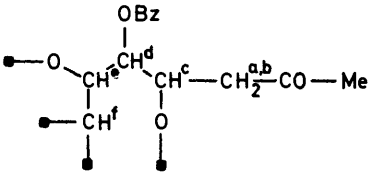


(XXV)

a; $R^1 = Bz, (R^2)_2 = Me_2C$
b; $R^1 = R^2 = Bz$

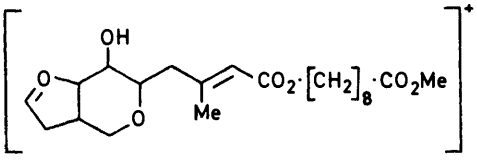


(XXVI)

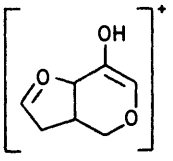


On treatment with benzoyl chloride in pyridine, (XXIVa) formed a tribenzoate (XXIVd), which was converted into its methyl ketone derivative (XXVb), $C_{36}H_{38}O_9$, by the above procedure. ^1H N.m.r. double resonance experiments on (XXVb) were again hampered by the clustering of signals. However, it was shown that the methyl group resonating at τ 8.74 was coupled to one of the protons (H^n) (τ 4.89) on a carbon atom bearing a benzoate group; the latter signal sharpened to a doublet ($J_{m,n}$ 8 Hz) on irradiation at the former position. This indicated that the C-13 hydroxy-group was not implicated in the acid-catalysed rearrangement of (I), which, if we assume that no carbon–carbon bonds have

been broken, must therefore involve the displacement of one of the epoxide carbon–oxygen bonds by the C-7 hydroxy-group. Dreiding models showed that both modes of cyclisation are possible, if the relative stereochemistry deduced for the pyran ring in (I) is correct, leading to the bicyclic compounds (XXIVa) and (XXVI). Structure (XXIVa) was favoured on the grounds that it is more likely to form a stable acetonide. This was supported by the mass spectrum of the acetonide (XXIVb), $M^{+\cdot}$ 554, which in addition to the normal cleavage of the αβ-unsaturated ester side chain (*m/e* 285) showed peaks at *m/e* 411 and 141 ascribed to the ions (XXVII) and (XXVIII).



(XXVII)



(XXVIII)

These observations firmly establish the structure of methyl pseudomonate A as (IIa) and hence of pseudomonic acid A as (I), and are in full agreement with the results of an *X*-ray crystallographic study [15] which has just been completed on a derivative of (I). Pseudomonic acid A is not readily classified on a structural basis with any of the known antibiotic groups.[16] Perhaps the nearest relatives are the polycyclic polyether monocarboxylic acids such as nigericin,[17] monensin,[18] and alborixin.[19]

Structurally, pseudomonic acid A contains several unusual features, including the 9-hydroxynonanoic acid residue, which are of biosynthetic interest. These are discussed in the accompanying paper.[4]

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage microscope. Unless otherwise stated, i.r. spectra were measured for solutions in chloroform, u.v. spectra in ethanol, and 60 and 100 MHz ^{1}H n.m.r. spectra in deuteriochloroform (tetramethylsilane as internal reference). Mass spectra were recorded at 70 eV with an A.E.I. MS9 high resolution spectrometer. Optical rotations were measured for solutions in chloroform at room temperature with a Perkin-Elmer 141 polarimeter. Evaporation refers to evaporation under diminished pressure. Light petroleum refers to the fraction with b.p. 60—80 °C unless otherwise stated.

Separations by column chromatography were carried out with Hopkin and Williams MFC grade silica (100—200 mesh) and Amberlite XAD-2 polystyrene resin. Thin-layer (t.l.c.) and preparative layer chromatography (p.l.c.) were performed on silica gel GF_{254} (Merck). T.l.c. spots were developed by spraying with 6N-H_2SO_4 followed by heating at 110 °C. During laboratory isolation processes the appropriate combination of fractions was determined by t.l.c.

Culture of Pseudomonas fluorescens (N.C.I.B. 10586) (with G. Banks and C. Wright) *and Isolation of Methyl Pseudomonate A* (IIa).—The bacterium was cultured on malt agar and incubated at 30 °C for 24 h. A heavy loop was used to inoculate each of two flasks containing medium (100 ml) of the following composition (% w/v): corn steep liquor (1.0) $MgSO_4,H_2O$ (0.037 5), KH_2PO_4 (0.04), Na_2HPO_4 (0.065), KCl (0.05), $MnCl_2,4H_2O$ (0.000 3), and silicone antifoam (0.01% v/v), which were shaken for 18 h at 30 °C. The combined cultures (*ca.* 150 ml.) were used to inoculate a 50 l fermenter containing the same medium, which was agitated at 24 °C during 18 h. An aliquot portion (10 l) of the small fermenter served as inoculum for a 3 000 l fermenter containing the same medium which was agitated at 24 °C for 24 h; maximum antibiotic production was then attained. Barium chloride (0.5% w/v) was added. After 18 h the cells and non-active material were removed by centrifugation. The supernatant was partitioned into isobutyl methyl ketone (0.2 vol) at pH 4.5; the extract was washed with water and the solvent removed *in vacuo* to leave *ca.* 5 l of solution. At this point neutral material was retained in the isobutyl methyl ketone layer on partitioning against water at pH 7.5. The aqueous phase was either freeze-dried to give a mixture of sodium salts (*ca.* 50 g) which could be conveniently stored for several months without loss of activity, or re-extracted into isobutyl methyl ketone at pH 4.5 to give the free acids. After removal of solvent, the acids were dissolved in methanol and treated with an excess of ethereal diazomethane overnight. Removal of solvent afforded a mixture of methyl esters.

Purification of Ammonium Salts on Amberlite XAD-2 Polystyrene Resin.—The mixed sodium salts (6 g) were converted into the free acids and the latter re-extracted into water (30 ml) as their ammonium salts (NH_4OH). A column packed with Amberlite XAD-2 resin (1.2 kg) was washed with water until chloride-free. The ammonium salt concentrate was introduced and eluted with a linear gradient produced by adding methanolic 0.01N-ammonia (4 l) to aqueous 0.01N-ammonia (4 l); 80 ml fractions were collected. Fractions 1—13 contained a light oil which comprised mainly 3-hydroxydecanoic acid. Fractions 14—44 afforded a residue which was dissolved in methanol and treated overnight with an excess of diazomethane in ether to give at least two components (t.l.c.). After removal of solvent the methyl esters (2.55 g) were separated by successive development on p.l.c. with propan-2-ol–chloroform (5 : 95 and 10 : 90). The band at R_F 0.35 afforded methyl pseudomonate B (0.16 g). The band at R_F 0.50 afforded a thick gum (1.28 g) which crystallised from benzene–light petroleum to give *methyl pseudomonate A* (IIa), m.p. 76.5—78°, $[\alpha]_D$ −9° (*c* 1.5), $\lambda_{max.}$ 221.5 nm (ε 13 400), $\nu_{max.}$ (CCl_4) 3 600—3100 (OH), 1 740 (CO_2Me), 1 715, 1 650 (αβ-unsaturated ester), 1 220, 1 150, and 1 050 cm^{-1}, τ 9.09 (3 H, d, *J* 7 Hz), 8.81 (3 H, d, *J* 6.5 Hz), 8.72 (*ca.* 10 H, s), 7.86br (3 H, s), 7.75 (2 H, t, *J* 7 Hz), 7.50 (1 H, s, OH), 7.45—7.20 (2 H, m), 6.80—6.00 [11 H, including τ 6.42 (3 H, s, OMe) and τ 6.00 (2 H, t, *J* 7 Hz)], and 4.32br (1 H, s),

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m/e 514 (1.5%, $M^{+\cdot}$, $C_{27}H_{46}O_9$), 496, 483, 478, 465, 452, 434, 412 (Found: 412.246 1. $C_{22}H_{36}O_7$ requires 412.247 3), 366, 350, 327, 309, 299, 291, 283, 281, 270 (Found: 270.181 6. $C_{15}H_{26}O_4$ requires 270.183 1), 267, 227 (Found: 227.127 9. $C_{12}H_{19}O_4$ requires 227.128 3), 209, 183, 141, 129, 125, 111, 97, 96, 95, 83, 82, 81, 71, 69, 55, 43, and 41 (Found: C, 62.8; H. 8.9. $C_{27}H_{46}O_9$ requires C, 63.0; H, 9.0%). In later isolation work (11a) could be isolated directly by repeated crystallisation of the crude methyl esters or by column chromatography on silica gel MFC.

The p-*Bromophenacyl Ester* (IId).—The sodium salt mixture (3.7 g) was dissolved in water (15 ml), a solution of *p*-bromophenacyl bromide (2 g) in dimethylformamide (6 ml) added, and the whole kept overnight at room temperature. The mixture was poured into water and extracted into 95 : 5 ether–ethanol. The extract was washed with water, dried, and evaporated. The product (3.6 g) was chromatographed (p.l.c.) as described above. The band at at R_F 0.5 afforded the oily p-*bromophenacyl ester* (IId) (1.5 g), $\nu_{max.}$ 3 650—3 150 (OH), 1 745, 1 720, 1 700, 1 650, 1 250, 1 150, and 970 cm^{-1}, τ 9.11 (3 H, d, *J* 7 Hz), 8.83 (3 H, d, *J* 6.5 Hz), 8.69 (*ca* 10 H, s), 7.85br (3 H, s), 7.56 (2 H, t, *J* 7 Hz), 7.40—7.20 (2 H, m), 6.70—5.90 [8 H, including τ 5.98 (2 H, t, *J* 7 Hz)], 4.80 (2 H, s, phenacyl), 4.34br (1 H, s), and 2.38 (4 H, q, aromatic) (Found: C, 58.1; H, 6.9. $C_{34}H_{49}BrO_{10}$ requires C, 58.5; H, 7.0%).

Methyl Pseudomonate A Triacetate (IIb).—Methyl pseudomonate A (446 mg) was treated with acetic anhydride (1 ml) in pyridine (3 ml) at room temperature overnight. The mixture was poured into aqueous 5% sodium hydrogen carbonate. The product was extracted into chloroform and the solution washed with water, dried, and evaporated to afford the *triacetate* (IIb) as an oil (560 mg), $[\alpha]_D$ −1° (*c* 2), $\lambda_{max.}$ 221 nm (ε 14 000), $\nu_{max.}$ 1 745, 1 715infl, 1 650, 1 250, and 1 150 cm^{-1}, τ 9.06 (3 H, d, *J* 7 Hz), 8.77 (3 H, d, *J* 6.5 Hz), 8.05, 7.99, 7.94 (3 × 3 H, s, acetates), 7.86 (3 H, d, *J* 1 Hz), 6.40 (3 H, s, OMe), 6.00 (2 H, t, *J* 6 Hz), 5.31 (1 H, dd, *J* 3 and 9.5 Hz), 5.07 (1 H, quint, *J* 6 Hz), 4.78 (1 H, t, *J* 3 Hz), and 4.37br (1 H, s); *m/e* 640 ($M^{+\cdot}$, $C_{33}H_{52}O_{12}$), 609, 580, 549, 520, 489, 460, 453, 452, 393, 392, 371, 333, 332, 311, 273, 272, 251, 191, 157, and 97.

Methyl Pseudomonate A Tribenzoate (IIc).—Methyl pseudomonate A (257 mg) was treated with benzoyl chloride (1 ml) in pyridine (2 ml) at room temperature, overnight. After the usual work-up the product was purified by p.l.c. (chloroform). The band at R_F 0.5 furnished the *tribenzoate* (IIc) (465 mg) as an oil, $[\alpha]_D$ −11° (*c* 1), $\nu_{max.}$ 1 740, 1 720, 1 650, 1 600, 1 490, 1 270, and 1 150 cm^{-1} (Found: C, 69.5; H, 7.3. $C_{48}H_{58}O_{12}$ requires C, 69.7; H, 7.1%).

The Dihydro-derivatives (IVa *and* b).—Methyl pseudomonate A (40 mg) in ethanol was hydrogenated over platinum oxide (6 mg) until uptake ceased (1 h). After filtration through Celite and evaporation the oily product was purified by p.l.c. [propan-2-ol–chloroform (1 : 9)]. The band at R_F 0.3 afforded *methyl dihydropseudomonate A* (IVa) as an oil, showing u.v. end absorption only, $\nu_{max.}$ 3 600—3 100 (OH) and 1 740 cm^{-1}, τ 9.08 (6 H, d, *J* 7 Hz), 8.81 (3 H, d, *J* 7 Hz), 8.72 (*ca.* 10 H, s, methylene), 7.26 (2 H, m), 6.41 (3 H, s, OMe), and 6.00 (2 H, t, *J* 6 Hz) (Found: C, 62.7; H, 9.2. $C_{27}H_{48}O_9$ requires C, 62.8; H, 9.4%).

The oily *dihydro-triacetate* (IVb) (50 mg), similarly obtained from the triacetate (IIb) (52.5 mg), showed u.v. end absorption only, $\nu_{max.}$ 1 740 and 1 250 cm^{-1}, τ 9.06 (6 H, d, *J* 7 Hz), 8.75 (3 H, d, *J* 7 Hz), 8.72 (*ca.* 10 H, s, methylene), 7.32 (2 H, m), 8.02, 7.98, and 7.94 (3 × 3 H, s, acetates), 6.40 (3 H, s, OMe), 6.01 (2 H, d, *J* 6.5 Hz), 5.32 (1 H, dd, *J* 3 and 9.5 Hz), 5.07 (1 H, quint, *J* 6 Hz), and 4.82 (1 H, t, *J* 3 Hz), *m/e* ($M^{+\cdot}$ 642 not seen) 611 ($M^{+\cdot}$—OMe), 583, 582, 523, 522, 463, 462, 455, 413, 395, 353, 335, 293, 275, 269, 233, 157, and 97.

Reduction of the Methyl Dihydro-ester (IVa) *with Lithium Aluminium Hydride.*—The methyl ester (IVa) (250 mg) and lithium aluminium hydride (250 mg) in tetrahydrofuran were were heated under reflux during 3 h. The mixture was decomposed with saturated aqueous ammonium chloride and filtered. After evaporation, the product was separated by p.l.c. [six elutions; chloroform–propan-2-ol (85 : 15 v/v)]. The three major bands were eluted with propan-2-ol. The band at R_F 0.95 afforded nonane-1,9-diol (VI) (200 mg) as needles, m.p. 46° (from light petroleum–ether); bisphenyl carbamate, needles, m.p. 168—169° (from ethanol) (lit.,[1] m.p.s 42.5—44.5 and 171.5—172.5°, respectively). The bands at R_F 0.30 and 0.25, although homogeneous on t.l.c., each contained at least two components as seen from the multitude of methyl signals in their n.m.r. spectra, and were not further investigated.

Reaction of Methyl Pseudomonate A with Potassium Hydroxide in Methanol.—Several attempts to hydrolyse cleanly the αβ-unsaturated ester group were made. The following conditions gave the best results. Methyl pseudomonate A (IIa) (257 mg) was dissolved in methanolic 0.5N-potassium hydroxide (5 ml) and left at room temperature for 24 h. The mixture was poured into water (30 ml), dilute hydrochloric acid was added (to pH 4), and the solution was extracted with butan-1-ol (3 × 30 ml). The combined extracts were evaporated and the residue treated with an excess of ethereal diazomethane, overnight. T.l.c. indicated three major components. The mixture was separated by double elution on p.l.c. [propan-2-ol–chloroform (5 : 95)]. The band at R_F 0.7 afforded the oily methyl 9-hydroxynonanoate (Vc) (51 mg), $\nu_{max.}$ 3 610 (OH) and 1 730 cm^{-1} (ester), τ 8.72 (10 H, s), 8.48 (2 H, t, *J* 7 Hz), 8.17 (1 H, s, OH), 7.74 (2 H, t, *J* 7 Hz), 6.43 (2 H, t, *J* 7 Hz), and 6.40 (3 H, s, OMe). When, instead of being treated with diazomethane, the extracted acids were converted into their sodium salts and treated with an excess of *p*-bromophenacyl bromide in aqueous dimethylformamide, then subjected to p.l.c., the p-*bromophenacyl ester* (Vb), m.p. 77.5—78° (needles from ethanol) was obtained, $\nu_{max.}$ 3 610 (OH), 1 745, 1 710, and 1 590 cm^{-1}, *m/e* 372/370 ($M^{+\cdot}$), 354/352, 285/283, 271/269, 258/256, 217/215, 200/198, 186/184, 185/183, and 157/153, τ 8.70 (8 H, s), 8.50 (5 H, m), 7.58 (2 H, t, *J* 7 Hz), 6.43 (2 H, t, *J* 6 Hz), 5.82 (2 H, s), and 2.36 (4 H, q) (Found: C, 55.1; H, 6.4. $C_{17}H_{23}BrO_4$ requires C, 55.0; H, 6.2%).

The two polar methyl esters, R_F 0.4 (92 mg) and 0.33 (65 mg), were each treated with benzoyl chloride in pyridine, at room temperature overnight. After the usual work-up, the benzoylated derivative of the component R_F 0.4 (93.6 mg) showed τ 8.90 (3 H, d, *J* 7.5 Hz), 8.68 (3 H, d, *J* 6 Hz), 7.93 (3 H, s), 6.39 (3 H, s, OMe), 4.90—4.55 (4 H), and 2.7—1.9 (20 H, aromatic). The benzoylated derivative of the component R_F 0.33 (81 mg) had τ 9.19 (3 H, d, *J* 7.5 Hz), 8.74 (3 H, d, *J* 6.5 Hz), 7.83 (3 H, s), 6.39 (3 H, s, OMe), 5.66 (1 H, dd, *J* 5 and 9 Hz), 5.10—4.7 (3 H), 4.31br (1 H, s), and 2.7—1.9 (15 H, aromatic). Neither compound was characterised further and both remain unidentified.

The Methyl Ketones (VIIIa—c) *and* (XIV).—The procedure for the preparation of these derivatives is common to all and is only reported in full for (VIIIb). To a solution of methyl pseudomonate A tribenzoate (11c) (261 mg) in pyri-

dine (5 ml) was added osmium tetraoxide (100 mg, 1.2 equiv.) and the resulting solution was set aside for 2 h at ambient temperature. After cooling to 0 °C sodium disulphite (0.4 g) in water (2 ml) was added over 15 min, with vigorous stirring, and the whole warmed to room temperature over 2 h. The mixture was poured into water and the product extracted into chloroform. The extract was washed with water, dried, and evaporated to give the oily diol (VIIb) (260 mg), $[\alpha]_D$ −26° (*c* 1), $\nu_{max.}$ 3 500 (OH) 1 735, 1 725, 1 275, and 1 120 cm^{-1}. The diol, without further characterisation, was dissolved in ethanol (25 ml) containing water (5 ml). A solution of sodium periodate (250 mg) in water (1 ml) was added during 10 min. After 1 h the mixture was poured into water and extracted into chloroform. The extract was washed with water, dried, and evaporated leaving an oily mixture of (VIIIb) and (IX). The products were separated by double elution on p.l.c. (chloroform). The band at R_F 0.4 gave 8-*methoxycarbonyloctyl glyoxylate* as an oil (84 mg), $\nu_{max.}$ 1 745 cm^{-1}, τ 8.66 (10 H, s), 7.71 (2 H, t, *J* 7 Hz), 6.37 (3 H, s, OMe), 5.81 (2 H, t, *J* 7 Hz), and −0.28 (1 H, s, CHO) (Found: C, 58.8; H, 8.2. $C_{12}H_{20}O_5$ requires C, 59.0; H 8.2%); *semicarbazone*, m.p. 164—165.5° (plates from ethanol) (Found: C, 51.6; H, 7.6; N, 13.8. $C_{13}H_{23}N_3O_5$ requires C, 51.8; H, 7.7; N, 14.0%). The band at R_F 0.55 afforded the oily 3,4-*bisbenzoyloxy*-5-(5-*benzoyloxy*-2,3-*epoxy*-4-*methylhexyl*)*tetrahydropyran*-2-*ylacetone* (VIIIb) (106 mg), $\nu_{max.}$ 1 745, 1 270, and 1 120 cm^{-1}, τ 8.92 (3 H, d), 8.58 (3 H, d), 7.90 (3 H, s), 7.48 (H^a, dd), 7.28 (H^b, dd), 7.19 (H^k, finely split triplet), 7.07 (H^l, dd), 6.25 (H^g, dd), 5.95 (H^h, dd), 5.52 (H^c, octet), 4.88 (H^d, dd), 4.79 (H^n, quint), 4.38 (H^e, t), and 3.0—1.80 (15 H, m, aromatic), $J_{a,b}$ 15, $J_{a,c}$ 4, $J_{b,c}$ 8, $J_{c,d}$ 10, $J_{d,e}$ 3, $J_{e,f}$ 3, $J_{f,g}$ 1, $J_{f,h}$ 2, $J_{i,k}$ 6, $J_{j,k}$ 6, $J_{k,l}$ 2, $J_{l,m}$ 7.5, $J_{m,n}$ 6, $J_{m,Me}$ 7, and $J_{n,Me}$ 6 Hz (Found: C, 70.1; H, 6.3. $C_{36}H_{38}O_9$ requires C, 70.4; H, 6.3%).

Methyl pseudomonate A triacetate (530 mg) afforded (IX) (200 mg) and the oily *methyl ketone triacetate* (VIIIa) (331 mg), R_F 0.3 (two elutions with chloroform), $[\alpha]_D$ −17° (*c* 2), $\nu_{max.}$ 1 745, 1 230, and 1 120 cm^{-1}, τ 9.03 (3 H, d), 8.75 (3 H, d), 7.86 (3 H, s), 7.60 (H^a, dd), 7.40 (H^b, dd), 7.35 (H^k, finely split triplet), 7.31 (H^l, dd), 6.38 (H^g, dd), 6.10 (H^h, dd), 5.84 (H^c, octet), 5.28 (H^d, dd), 5.06 (H^n, quint), and 4.76 (H^e, t), $J_{a,b}$ 16, $J_{a,c}$ 5, $J_{b,c}$ 8, $J_{c,d}$ 10, $J_{d,e}$ 3, $J_{e,f}$ 3, $J_{f,g}$ 1.5, $J_{f,h}$ 3, $J_{i,k}$ 6, $J_{j,k}$6, $J_{k,l}$ 2, $J_{l,m}$ 8, $J_{m,n}$ 6, $J_{m,Me}$ 7, and $J_{n,Me}$ 6.5 Hz (Found: C, 59.0; H, 7.5. $C_{21}H_{32}O_9$ requires C, 58.9; H, 7.5%).

The acetonide acetate (IIg) (1.58 g) furnished the *methyl ketone acetonide acetate* (VIIIc) (794 mg), R_F 0.45 [propan-2-ol–chloroform (1 : 99)], $\nu_{max.}$ 1 740, 1 720, 1 380, 1 370, and 1 240 cm^{-1}, τ 9.07 (3 H, d), 8.74 (3 H, d), 8.66 and 8.52 [2 × 3 H, s, $Me_2C(O)$·O], 7.99 (6 H, s, two acetates), 7.87 (6 H, s, one acetate + MeCO), 7.42 (2 H, m), 7.39 (H^l, dd), 7.27 (H^k, finely split t), and 5.07 (H^n, quint), $J_{i,k}$ 6, $J_{j,k}$ 6, $J_{k,l}$ 2.2, $J_{l,m}$ 7.5, $J_{m,n}$ 6, $J_{m,Me}$ 6, and $J_{n,Me}$ 7 Hz, *m/e* 380 (M^+), 369, 326, 309, 269, 241, 209, 181, 108, 85, 83, and 69 (Found: C, 62.8; H, 8.9. $C_{20}H_{32}O_7$ requires C, 63.1; H, 8.8%).

The bicyclic tribenzoate (XIIIa) (650 mg) yielded the crystalline *bicyclic methyl ketone tribenzoate* (XIV) (450 mg), m.p. 138.5—140° (needles from ethanol), $[\alpha]_D$−4° (*c* 1.5), R_F 0.60 (two elutions with chloroform), $\nu_{max.}$ 1 725, 1 715, 1 605, 1 500, 1 280, 1 180, and 1 120 cm^{-1}, τ 8.83 (3 H, d), 8.70 (3 H, d), 8.12 (H^m, m), 8.1—7.9 (H^i and H^j, m), 7.92 (3 H, s), 7.80 (H^f, m), 7.55 (H^a, dd), 7.33 (H^b, dd), 6.53 (H^n, quint), 6.31 (H^g, dd), 5.99 (H^h, dd), 5.94 (H^k, dd), 5.59 (H^c, oct), 4.95 (H^l, dd), 4.85 (H^d, dd), 4.34 (H^e, t), and 2.90—1.80 (15 H, m, aromatic), $J_{a,b}$ 12, $J_{a,c}$ 4, $J_{b,c}$ 8.5, $J_{c,d}$ 10, $J_{d,e}$ 3, $J_{e,f}$ 3, $J_{f,g}$ 1, $J_{f,h}$ 1, $J_{g,h}$ 12, $J_{i,k}$ 6, $J_{j,k}$ 6, $J_{k,l}$ 5, $J_{l,m}$ 3, $J_{m,n}$ 7, $J_{m,Me}$ 6.5, and $J_{n,Me}$ 7 (Found: C, 70.1; H, 6.2. $C_{36}H_{38}O_9$ requires C, 70,4; H, 6.3%).

The Acetonide (IIe).—Methyl pseudomonate A (IIa) (200 mg) and toluene-*p*-sulphonic acid (one crystal) were dissolved in 2,2-dimethoxypropane (5 ml), and the solution was set aside for 2 h at ambient temperature. Sodium hydrogen carbonate (50 mg) was added and stirring continued for ½ h, after which the mixture was filtered and evaporated. The oily residue was purified by p.l.c. (6 : 94 propan-2-ol–chloroform) to give the oily *acetonide* (IIe) (187 mg), R_F 0.55, $[\alpha]_D$ −25° (*c* 1), $\lambda_{max.}$ 221 nm (ε 13 100), $\nu_{max.}$ 3 620, 1 740, 1 715, 1 650, 1 220, and 1 150 cm^{-1}, τ 9.09 (3 H, d, *J* 7 Hz), 8.87 (3 H, d, *J* 7 Hz), 8.70 and 8.55 [2 × 3 H, s, $Me_2C(O)$·O], 7.86 (3 H, s), 7.38 (1 H, dd, *J* 7.5 and 2 Hz), 7.22 (1 H, finely split t, *J* 6 and 2 Hz), 6.41 (3 H, s, OMe), 6.00 (2 H, t, *J* 6 Hz), and 4.34br (1 H, s), *m/e* 554 ($M^{+\cdot}$), 538, 523, 510, 496, 480, 465, 367, 309, 284, 270, 227, 226, 209, 109, 97, 83, 82, and 81 (Found: C, 64.9; H, 9.0. $C_{30}H_{50}O_9$ requires C, 65.0; H, 9.1%); 4-(p-*nitrophenylazo*)*benzoate* (IIf), m.p. 72—74° (red needles from ethanol) (Found: C, 63.4; H, 6.8; N, 4.9. $C_{43}H_{57}N_3O_{12}$ requires C, 63.9; H, 7.1; N, 5.2%).

The Acetonide Ketone (IIe, C-13 ketone).—The acetonide (IIe) (100 mg) was stirred with Cornforth's reagent (10 ml) during 48 h at room temperature. The mixture was poured into water and the product extracted into ether. After washing with water and drying the extract was evaporated. The product was purified by p.l.c. [propan-2-ol–chloroform (4 : 96)]. The band at R_F 0.60 afforded the oily *acetonide ketone* (IIe, C-13 ketone) (81 mg), $[\alpha]_D$ −49° (*c* 1.1), $\lambda_{max.}$ 220.5 nm (ε 14 900), $\nu_{max.}$ 1 740, 1 720, 1 715, 1 650, 1 220, 1 150, and 1 060 cm^{-1}, τ 8.88 (3 H, d, *J* 7 Hz), 8.86 (3 H, d, *J* 6.5 Hz), 8.71 (*ca.* 10 H, s), 8.68 and 8.53 [2 × 3 H, s, $Me_2C(O)$·O], 7.86 (3 H, s), 7.82 (3 H, s, acetate), 7.26 (1 H, dd, *J* 7.5 and 2.2 Hz), 7.16 (1 H, finely split t, *J* 6 and 2.2 Hz), 6.42 (3 H, s, OMe), 6.00 (2 H, t, *J* 6.5 Hz), 4.92 (1 H, m), and 4.33br (1 H, s), *m/e* 552 ($M^{+\cdot}$), 534, 510, 494, 476, 463, 365, 349, 283, 282, 270, 225, 207, 197, 97, 85, 83, 82, and 81 (Found: C, 55.1; H, 8.7. $C_{30}H_{48}O_9$ requires C, 65.2; H, 8.8%).

The Acetonide Acetate (IIg).—The acetonide (IIe) (302 mg) was treated with acetic anhydride (1 ml) in pyridine (2 ml) overnight at room temperature. The usual work-up gave the acetonide acetate (IIg) (316 mg) as an oil, $[\alpha]_D$ −23° (*c* 1), $\lambda_{max.}$ 221.5 nm (ε 14 500), $\nu_{max.}$ 1 745, 1 725, 1 650, 1 245, and 1 150 cm^{-1}, τ 9.09 (3 H, d, *J* 7 Hz), 8.77 (3 H, d, *J* 6 Hz), 8.69 and 8.35 [2 × 3 H, s, $Me_2C(O)$·O], 7.86 (3 H, d, *J* 1 Hz), 7.39 (1 H, dd, *J* 8 and 2 Hz), 7.27 (1 H, finely split t, *J* 6 and 2 Hz), 6.42 (3 H, s, OMe), 6.00 (2 H, t, *J* 6 Hz), 5.08 (1 H, quint, *J* 6 Hz), and 4.34br (1 H, s), *m/e* 596 ($M^{+\cdot}$, $C_{32}H_{52}O_{10}$), 580, 565, 538, 480, 409, 327, 269, 267, 209, 149, 111, 109, 97, 95, 93, 82, and 81.

6-(6-*Acetonyl*-4,5-*isopropylidenedioxytetrahydropyran*-3-*yl*)-4,5-*epoxy*-3-*methylhexan*-2-*one* (XIX).—The methyl ketone acetonide acetate (VIIIc) (25 mg) was dissolved in methanolic 5% potassium hydroxide and kept for 1 h at ambient temperature. The mixture was poured into water and extracted into ether. The extract was evaporated and the product was purified by p.l.c. [propan-2-ol–chloroform (6 : 94)]. The band at R_F 0.5 afforded the oily hydroxy-acetonide (XX) (20 mg), $\nu_{max.}$ 3 480 (OH) and 1 720 cm^{-1}, which was not further characterized. The hydroxy-acetonide (20 mg) was stirred at room temperature overnight with Cornforth's reagent (0.5 ml). The mixture was poured into aqueous 5% sodium hydrogen carbonate and the product extracted into chloroform. The extract was evaporated

and the product subjected to p.l.c. [propan-2-ol–chloroform (3 : 97)]. The band at R_F 0.45 afforded the oily diketone (XIX) (17 mg), $\nu_{max.}$ 1 720 and 1 710 cm^{-1}, τ 8.87 (3 H, d, J 7 Hz), 8.69 (3 H, d, J 6 Hz), 8.52 (6 H, s), and 7.82 (6 H, s), *m/e* 340 ($M^{+\cdot}$, $C_{18}H_{28}O_6$), 325, 282, 264, 221, 154, 111, 109, 95, 83, 81, and 69.

Reaction of the Diketone (XIX) *with Base*.—The diketone (XIX) (15 mg) was stirred with potassium t-butoxide (15 mg) in t-butyl alcohol (0.5 ml) at room temperature for 1 h. [The hydroxyacetonide (XX) was unchanged when subjected to these conditions.] T.l.c. [propan-2-ol–chloroform (3 : 97)] showed one major product, R_F 0.35, which quenched the fluorescent indicator at 254 nm. The reaction mixture was spotted directly on p.l.c. After elution with the same solvent, the band at R_F 0.35 afforded the major product, tentatively formulated as the αβ-unsaturated ketone (XXI), as an unstable oil, $\nu_{max.}$ 3 610 (OH), 1 720 (MeCO), 1 675 (αβ-unsaturated C:O), and 1 380, 1 370, and 1 355 [$Me_2C(O)\cdot O$], which was not further characterized.

Treatment of Methyl Pseudomonate A Tribenzoate (IIc) *with Perchloric Acid*.—To methyl pseudomonate A tribenzoate (IIc) (198 mg) in ethyl acetate (5 ml), at room temperature, was added aqueous 60% perchloric acid (0.5 ml), with stirring. The reaction was monitored by t.l.c. ($CHCl_3$) which showed the rapid formation of a polar compound, R_F 0.05, followed equally quickly by the formation of a product, R_F 0.55, slightly less polar than (IIc). The reaction was complete after 5 min. The mixture was poured into aqueous 5% sodium hydrogen carbonate and extracted with chloroform. The extract was evaporated and the product was purified by p.l.c. [propan-2-ol–chloroform (2 : 98)]. The band at R_F 0.65 afforded the *bicyclic tribenzoate* (XIIIa) (161 mg), which crystallised as needles from benzene–light petroleum (1 : 5), m.p. 122—123°, $[\alpha]_D$ −2° (*c* 1), $\lambda_{max.}$ 281 (ε 2 020), 273.5 (2 620), 268 (2 260), and 227.5 nm (48 250), $\nu_{max.}$ 1 740, 1 725, 1 650, 1 605, 1 275, and 1 150 cm^{-1}, τ 8.82 (3 H, d, J 7 Hz), 8.74 (*ca.* 10 H, s, methylene), 8.71 (3 H, d, J 6 Hz), 7.86 (3 H, d, J 1 Hz), 6.52 (1 H, quint, J 6 Hz), 6.41 (3 H, s, OMe), 6.05 (2 H, t, J 6.5 Hz), 4.93 (1 H, dd, J 6 and 3 Hz), 4.85 (1 H, dd, J 10 and 3 Hz), 4.37br (1 H, s), 4.34 (1 H, t, J 3 Hz), and 2.90—1.90 (15 H, m, aromatic) (Found: C, 6.99; H, 7.0. $C_{36}H_{48}O_{12}$ requires C, 69.6; H, H, 7.0%).

In a separate reaction which was quenched after 2 min the product was converted into the methyl ketone derivatives. After p.l.c., more polar component (15 mg), R_F 0.10 (chloroform), was isolated and tentatively assigned structure (XV) on the basis of its n.m.r. spectrum: τ 8.98 (3 H, d, J 7 Hz), 8.78 (3 H, d, J 6.5 Hz), 7.95 (3 H, s), 5.57 (1 H, oct, J 10, 7, and 5 Hz), 4.92 (1 H, dd, J 10 and 3 Hz) ,4.10 (1 H, dd, J 7 and 3 Hz), 4.36 (1 H, t, J 3 Hz), and 2.8—1.9 (15 H, m, aromatics). When re-subjected to the above conditions (XV) was rapidly cyclized to (XIV) (t.l.c.).

Treatment of Methyl Pseudomonate A Triacetate (IIb) *with Perchloric Acid*.—Methyl pseudomonate A triacetate (IIb) (114 mg) in ethyl acetate (4 ml) containing aqueous 60% perchloric acid (0.4 ml) was stirred at room temperature for 45 min. The reaction was complete after 30 min (t.l.c.). After work-up as described above, the two-component product was separated by p.l.c. [propan-2-ol–chloroform (1 : 99)]. The band at R_F 0.54 gave the bicyclic triacetate (XIIIb) (93 mg) as an oil, $[\alpha]_D$ +17° (*c* 1.5), $\nu_{max.}$ 1 745, 1 715, 1 655, 1 250, and 1 130 cm^{-1}, τ 8.92 (3 H, d, J 7 Hz), 8.75 (3 H, d, J 6.5 Hz), 8.70 (*ca.* 10 H, s), 8.05 (3 H, s), 7.95 (6 H, s, acetates), 7.84br (3 H, s), 6.63 (1 H, quint, J 6.5 Hz), 6.40 (3 H, s, OMe), 5.98 (2 H, t, J 6.5 Hz), 5.28 (1 H, dd, J 10 and 3 Hz), 5.22 (1 H, dd, J 5 and 3.5 Hz), 4.74 (1 H, t, J 3 Hz), and 4.34br (1 H, s), *m/e* 640 ($M^{+\cdot}$, $C_{33}H_{52}O_{12}$), 609, 580, 538, 520, 460, 453, 452, 311, 269, 241, 209, 191, 171, 157, 111, 97, 95, 83, 81, and 43.

The Methyl Pseudomonate A Isomer, Methyl 9-(4-{8-(1,3-Dihydroxy-2-methylbutyl)-5-hydroxy-3,7-dioxabicyclo[4.3.0]*-nonan-4-yl}-3-methylbut-2-enoyloxy)nonanoate* (XXIVa).—The crude acidic extract from a 3 000 l fermentation of *P. fluorescens* which had been kept at 4 °C for 48 h in an isobutyl methyl ketone–water emulsion at pH < 4 was treated with ethereal diazomethane (in excess). The oily methyl esters were chromatographed on silica gel (MFC) [elution successively with chloroform (2 l), 1%, 2%, 3%, and 4% propan-2-ol in chloroform (1.5 l), and 5%, 10%, and 20% propan-2-ol in chloroform (1 l)]. Fractions eluted in the range 10—20% propan-2-ol in chloroform were combined and evaporated. The oily residue (5 g) was further purified by p.l.c. [propan-2-ol–chloroform (1 : 9)]. The band at R_F 0.5 afforded the oily *methyl pseudomonate A isomer* (XXIVa) (3.4 g), $[\alpha]_D$ −9° (*c* 1.5), $\lambda_{max.}$ 223 nm (ε 11 300), δ_C 174.4 (s, C-1′), 166.5 (s, C-1), 155.1 (s, C-3), 118.4 (d, C-2), 82.1 (d), 76.1 (d), 75.7 (d), 70.2 (d), 69.2 (d), and 66.4 (d) (C-5—7, -10, -11, and -13), 64.2 (t, C-16), 64.0 (t, C-9′), 51.5 (q, OMe), 40.0 (t, C-4; d, C-12), 34.7 (t, C-9), 34.1 (t, C-2′), 32.9 (d, C-8), 29.1 (C-4′—6′), 28.7 (t, C-8′), 26.0 (t, C-7′), 24.9 (t, C-3′), 22.1 (q, C-14), 18.4 (q, C-15), and 10.8 (q, C-17) (d, t, and q: multiplicities in off-resonance spectrum) (Found: C, 62.6; H, 9.0. $C_{27}H_{46}O_9$ requires C, 63.0; H, 9.0%), chromatographically identical with (IIa).

The Acetonide (XXIVb).—The methyl pseudomonate A isomer (XXIVa) (1 g) in 2,2-dimethoxypropane (10 ml) containing a crystal of toluene-*p*-sulphonic acid was stirred for 1 h. After evaporation of the excess of dimethoxypropane, 150 mg of the oily residue was purified by p.l.c. [propan-2-ol–chloroform (4 : 96)]. The band at R_F 0.4 afforded the *acetonide* (XXIVb) (130 mg), $\lambda_{max.}$ 222.5 nm (ε 12 300), $\nu_{max.}$ (CCl_4) 3 590 (OH), 1 740 (ester,) 1 720, 1 650 (αβ-unsaturated ester), 1 225, 1 150, 1 090, and 870 cm^{-1} (Found: C, 64.8; H, 8.7. $C_{30}H_{50}O_9$ requires C, 65.0; H, 9.1%), *m/e* 554 (1%, $M^{+\cdot}$), 536, 523, 496, 478, 465, 411, 393, 367, 366, 285, 270, 227, 141, 111, 95, 82, 69, 55, and 43.

The Methyl Ketone Acetonide Benzoate (XXVa).—The unpurified acetonide derivative (above; 600 mg) was treated with benzoyl chloride (1 ml) in pyridine (10 ml) at room temperature for 4 h. After the usual work-up the product was purified by p.l.c. [propan-2-ol–chloroform (2 : 98)] to give the oily acetonide benzoate (XXIVc), R_F 0.6 (610 mg). This compound was not characterised but immediately converted by the procedure described for (VIIIb) into (IX) and the *methyl ketone acetonide benzoate* (XXVa), $C_{25}H_{34}O_7$, $\nu_{max.}$ 1 740, 1 720, 1 600, 1 490, and 1 150 cm^{-1}, τ 9.16 (3 H, d, J 7 Hz), 8.93 (3 H, d, J 6 Hz), 8.73 (6 H, s), 7.84 (3 H, s), 7.42 (H^a, dd), 7.32 (1 H, t), 7.08 (H^b ,dd), 6.10—6.85 (5 H), 6.72 (H^e, dd), 5.41 (H^c, oct), 4.85 (H^d, poorly defined dd), and 1.8—2.8 (5 H), $J_{a,b}$ 16, $J_{a.c}$ 6, $J_{b.c}$ 10, $J_{c.d}$ 1, $J_{d.e}$ 2.5, and $J_{e,f}$ 10 Hz (Found: C, 67.4; H, 7.7. $C_{25}H_{34}O_7$ requires C, 67.2; H, 7.6%).

The Methyl Ketone Tribenzoate (XXVb).—The methyl pseudomonate A isomer (XXIVa) (500 mg) was treated with benzoyl chloride (2 ml) in pyridine (10 ml) at room temperature overnight. After the usual work-up, the tribenzoate (XXIVd) was converted, by the above method, into (IX) and the *methyl ketone tribenzoate* (XXVb), which were separated by p.l.c. [propan-2-ol–chloroform (3 : 97)]. The

latter showed $\nu_{max.}$ 1 740, 1 720, 1 590, 1 490, 1 270, and 1 150 cm^{-1}, τ 9.22 (3 H, d, J 7 Hz), 8.74 (3 H, d), 7.85 (3 H, s), 7.46 (H^a, dd), 7.15 (H^b, dd), 6.29—6.70 (4 H), 5.06 (H^k, m), 4.89 (H^n, m), 4.82 (H^d, poorly defined dd), and 1.9—2.7 (15 H), $J_{a,b}$ 15, $J_{a,c}$ 5, $J_{b,c}$ 10, $J_{c,d}$ 1, $J_{d,e}$ 2.5, and $J_{n,Me}$ 8 Hz (Found: C, 70.0; H, 6.1. $C_{36}H_{38}O_9$ requires C, 70.4; H, 6.3%).

The Trimethyl Ether (IIh).—To dimethylacetamide (25 ml) at 0 °C was added, with stirring, sodium hydride [from a 60% suspension in paraffin oil (1.6 g) by repeated washing with dry ether]. After effervescence ceased, methyl iodide (3 ml) was added during 10 min. After 30 min, methyl pseudomonate A (IIa) (980 mg) was added in small amounts during 10 min. The mixture was stirred for 30 min and allowed to warm to room temperature over 2 h. The mixture was poured into ice–water and extracted with chloroform. The extract was washed with water, dried, and evaporated to give the oily *trimethyl ether* (IIh), (1.00 g), $[\alpha]_D$ −15° (*c* 1.7), $\nu_{max.}$ 1 730, 1 715, and 1 650 cm^{-1}, $\lambda_{max.}$ 220.5 nm (ε 7 250), τ 9.09 (3 H, d, J 7 Hz), 8.85 (3 H, d, J 6.5 Hz), 8.77 (*ca.* 10 H, s, methylene), 7.82br (3 H, s), 7.4—7.2 (2 H, m), 6.67, 6.60, and 6.52 (3 × 3 H, s, 3 × OMe), 6.34 (3 H, s, CO_2Me), 5.98 (2 H, t, J 7 Hz), and 4.30br (1 H, s) (Found: C, 64.7; H, 9.4. $C_{30}H_{52}O_9$ requires C, 64.7; H, 9.4%).

Reaction of the Trimethyl Ether (11h) *with Periodic Acid.*—To a stirred solution of anhydrous periodic acid (350 mg) in dry ether (150 ml) at room temperature was added a solution of the trimethyl ether (11 h) (800 mg) in dry ether (10 ml). After 1 h the mixture, containing the aldehydes (XXIIa) and (XXIIIa), was filtered through sand. To the filtrate was added a solution of 2,4-dinitrophenylhydrazine (800 mg) in ethanol (10 ml) and the whole was stirred overnight. The mixture was concentrated *in vacuo* and the product separated into two components by p.l.c. [benzene–chloroform (25 : 75)]. The band at R_F 0.5 afforded the *2,4-dinitrophenylhydrazone* of *3-methoxy-2-methylbutyraldehyde* (XXIIb) (351 mg), which crystallised from ethanol–light petroleum (1 : 9) as orange needles, m.p. 76—78°, $[\alpha]_D$ +6°, τ 8.78 (6 H, d, J 6.5 Hz), 7.34 (1 H, sext, J 6.5 Hz), 6.60 (3 H, s, OMe), 6.58 (1 H, quint, J 6.5 Hz), 2.46 (1 H, d, J 6.5 Hz), 2.14 (1 H, d, J 10 Hz), 1.78 (1 H, dd, J 10 and 2 Hz), 0.98 (1 H, d, J 2 Hz), and −1.00br (1 H, s, NH) (Found: C, 48.9; H, 5.7; N, 18.9. $C_{12}H_{16}N_4O_5$ requires C, 48.7; H, 5.4; N, 18.9%). The band at R_F 0.20 gave the 2,4-dinitrophenylhydrazone (XXIIIb) (781 mg) as an oil, $[\alpha]_D$ −11° (*c* 2.4), τ 6.88 (*ca.* 10 H, s), 7.80br (3 H, s), 6.66 and 6.61 (2 × 3 H, s, 2 × OMe), 6.34 (3 H, s, CO_2Me), 5.96 (2 H, t, J 6.5), 4.27br (1 H, s), 2.32 (1 H, t, 5.5 Hz), 2.13 (1 H, d, J 10 Hz), 1.73 (1 H, dd, J 10 and 2 Hz), 0.96 (1 H, d, J 2 Hz), and −1.10br (1 H, s) (Found: C, 56.6; H, 7.0; N, 9.1. $C_{30}H_{44}N_4O_{11}$ requires C, 56.6; H, 7.0; N, 8.8%).

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