Pseudomonic Acid. Part 3.[1] Structure of Pseudomonic Acid B

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Pseudomonic acid B, a minor antibiotic produced by *Pseudomonas fluorescens*, has been identified as 9-{4-[5-(2.3-epoxy-5-hydroxy-4-methylhexyl)-3.4,5-trihydroxytetrahydropyran-2-yl]-3-methylbut-2-enoyloxy}nonanoic acid from a comparison of spectral data of its derivatives with those of derivatives of pseudomonic acid A.

Pseudomonas fluorescens, when grown in submerged culture, produces a number of acidic, antimicrobially active substances[2,3] which, collectively, have good activity against Gram positive bacteria. We have presented evidence which firmly established structure (Ia) for the more abundant antibiotic in this family, pseudomonic acid A.[4] Here we present evidence that establishes structure (IIb) for the methyl ester of a minor isolate, conveniently termed pseudomonic acid B (IIa).

The molecular formula of methyl pseudomonate B (IIb), $C_{27}H_{46}O_{10}$, contains one more oxygen atom than that of methyl pseudomonate A (Ib). Methyl ester (1 740 cm^{-1}) and αβ-unsaturated ester (1 720 and 1 650 cm^{-1}) bands, as observed in the i.r. spectrum of (Ib), were also present. The u.v. spectrum, $\lambda_{max.}$ 221 nm (ε 13 000), provided additional evidence for the αβ-unsaturated ester linkage. The ^1H n.m.r. spectrum of (IIb) was very similar to that of (Ib), showing the presence of two secondary methyl groups [τ 9.12 (3 H, d, *J* 7 Hz) and 8.78 (3 H, d, *J* 6.5 Hz)], an olefinic methyl group [τ 7.83br (3 H, s)], methylene protons [τ 8.70 (*ca.* 10 H, s)], a methoxy-group [τ 6.40 (3 H, s)], and an olefinic proton [τ 4.31br (1 H, s)]. The presence of a two-proton multiplet at τ 7.0—7.4 implied that the epoxy-group of (Ib) was also present.

On acetylation with acetic anhydride in pyridine at ambient temperature, (IIb) formed a triacetate (IV), $C_{33}H_{52}O_{13}$, which showed hydroxy-absorbance ($\nu_{max.}$ 3 690 cm^{-1}) in its i.r. spectrum. This indicated that the extra oxygen was present in (IIa) as a tertiary hydroxy-group, which could only be located at either C-8 or C-12, provided that the integrity of the carbon framework of (Ia) is maintained in (IIa). The natural abundance, proton-noise-decoupled (p.n.d.) ^{13}C n.m.r. spectrum of (IIb) showed a total of 25 signals and was very similar to that of (Ib). Assignments were readily made by comparison with the spectrum of (Ib) [1] (Table).

Comparison of ^{13}C n.m.r. spectra of the esters (IIb) and (Ib)

Carbon	(IIb) †	(Ib)[1] †	Multiplicity ‡
1	166.8	166.8	s
2	117.7	117.6	d
3	156.4	156.7	s
4	42.9	42.9	t
5	70.2	74.8	d
6	72.9	69.0	d
7	74.6	70.4	d
8	72.0	39.5	s [d in (Ib)]
9	37.1	31.6	t
10	51.8	55.6	d
11	60.7	61.3	d
12	41.7	42.9	d
13	71.5	71.4	d
14	21.2	20.8	q
15	19.2	19.1	q
16	68.5	65.4	t
17	12.1	12.7	q
1′	174.4	174.4	s
2′	34.1	34.1	t
3′	24.9	24.9	t
4′	29.0	29.0	
5′	29.0	29.0	
6′	29.0	29.0	
7′	25.9	25.9	t
8′	28.7	28.7	t
9′	63.8	63.9	t
OMe	51.5	51.4	q

† P.p.m. to low field of Me_4Si; solvent $CDCl_3$. ‡ In off-resonance decoupled spectrum.

The signal of C-8 in (Ib) at 39.5 p.p.m. had disappeared in the spectrum of (IIb), and a new signal at 72.0 p.p.m. (C–O region) had appeared, which was a singlet in the

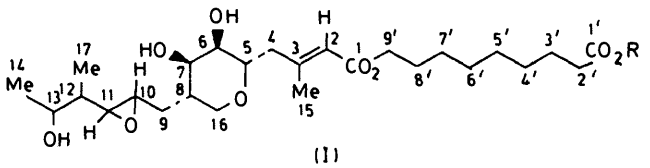
[1] Part 2, T. C. Feline, R. B. Jones, G. Mellows, and L. Phillips, preceding paper.

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

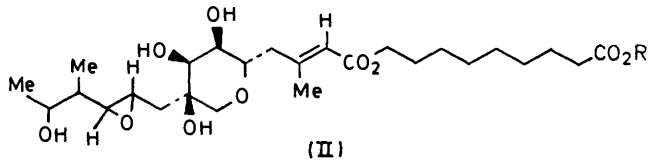
[3] A. T. Fuller, G. Mellows, M. Woolford, G. T. Banks, K. D. Barrow, and E. B. Chain, *Nature*, 1971, **234**, 416.

[4] E. B. Chain and G. Mellows, *J.C.S. Chem. Comm.*, 1974, 847; E. B. Chain and G. Mellows, *J.C.S. Perkin I*, 1976, 294.

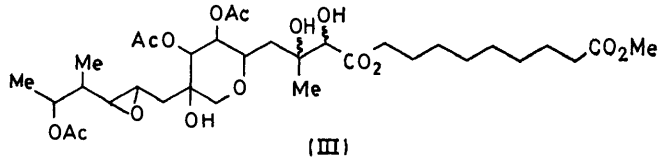
single-frequency off-resonance-decoupled spectrum. The tertiary hydroxy-group must therefore be at C-8. This



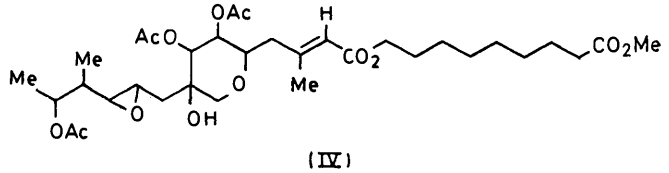
(I)
a; R = H
b; R = Me



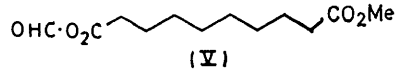
(II)
a; R = H
b; R = Me



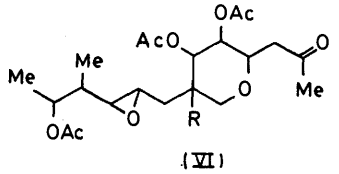
(III)



(IV)

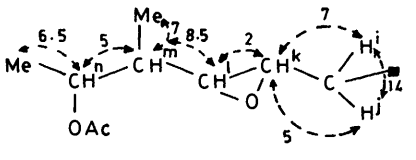


(V)



(VI)
a; R = OH
b; R = H

deduction was supported by the upfield shift of the C-16 signal by 3.1 p.p.m. and the alterations in the



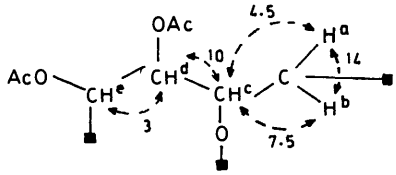
(VII)

chemical shifts of C-5, -6, and -7 (which could not be unambiguously assigned). The overlapping signals of C-4 and -12 in (Ib) were separated in the spectrum of (IIb), that of C-4 moving downfield by 1.2 p.p.m. The observed β, γ, and δ chemical shift changes of C-9, -10, and -11 (−5.5, +3.8, and −0.5 p.p.m.) were consistent with those expected on exchanging the C-8 hydrogen atom for a hydroxy-group.[5]

In order to confirm structure (IIb) for methyl pseudomonate B, the triacetate (IV) was treated with osmium tetraoxide in pyridine [4] followed by aqueous sodium hydrogen sulphite, generating the diol (III). The latter was not characterised, but immediately treated with sodium periodate in aqueous ethanol yielding 8-methoxycarbonyloctyl glyoxylate (V) and the hydroxy-methyl ketone triacetate (VIa), $C_{21}H_{32}O_{10}$, which were separated by p.l.c. The former was characterised as its semicarbazone, m.p. 162.5—164.5°, identical with the semicarbazone of (V) [4] derived from (Ib). The hydroxymethyl ketone triacetate showed hydroxy-absorption in its i.r. spectrum (3 610 cm^{-1}). Its ^1H n.m.r. spectrum was very similar to that of (VIb).[4]

Apart from small chemical shift changes, the main differences between the spectra of (VIa) and (VIb) were the appearance of the signal for the two methyleneoxy geminal protons (H^g and H^h) in the pyran ring as a singlet [τ 6.46; H^g and H^h signals having moved upfield by 0.08 and 0.36 p.p.m., respectively from their positions in the spectrum of (VIb)], and the loss of the 3 Hz coupling from the C-8 proton in (VIb) to H^e, the signal of which now appeared as a doublet ($J_{d,e}$ 3 Hz). Also, the signal of one of the C-9 protons (H^i), not clearly visible in the spectra of the methyl ketone derivatives of (Ib),[4] was observed in the spectrum of (VIa) at τ 8.21 as a double doublet ($J_{i,j}$ 14, $J_{i,k}$ 7 Hz), now being the A part of an ABX spin system, which lost the smaller coupling on irradiation at τ 7.06 (H^k). More extensive spin–spin decoupling experiments clearly established the part structures (VII) and (VIII). The value of $J_{j,k}$ (5 Hz) was obtained from the residual double doublet of H^k, seen on irradiation at τ 8.21 (H^i).

The attachment of the methyl ketone group to the carbon atom bearing H^a and H^b follows from the argument used in the formulation of (VIb).[4] There can be only one way of assembling part structures (VII) and (VIII), the carbon carrying the tertiary hydroxy-group, and the methyleneoxy group; this is depicted in (VIa). We have previously shown that the pyran ring in (VIb) [4] and (Ib) [6] adopts a chair (Cl) conformation with



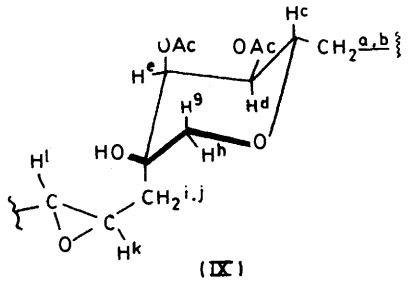
(VIII)

the substituents disposed as shown in (IX) (with H for OH). The values of $J_{c,d}$ and $J_{d,e}$ in (Ia) are identical

[5] J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, pp. 139—143.

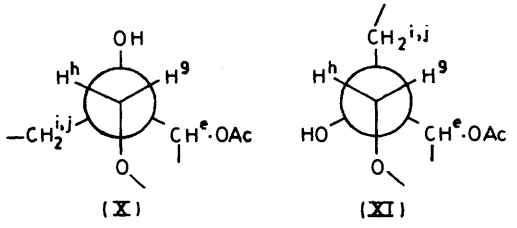
[6] M. Anteunis, A. De Bruyn, G. Mellows, and G. Verhegge, to be published elsewhere.

with the corresponding values for (VIb), indicating the similar disposition of the substituents at C-5, -6, and -7. Examination of the projection along the C(16)–C(8) bond (Dreiding models) indicates that the equivalence



(IX)

of chemical shifts for the ring methyleneoxy protons, H^g and H^h, is more likely when the C-8 hydroxy-group is equatorial (X) than when it is axial (XI). In the former situation, H^g and H^h occupy a more similar spatial disposition with respect to the ring oxygen and hydroxy-group. The 8-OH-equatorial configuration would also be expected, since (IIa) is probably formed, during the fermentation, by the enzymic hydroxylation of (Ia), which would be expected to proceed with retention of configuration.[7]



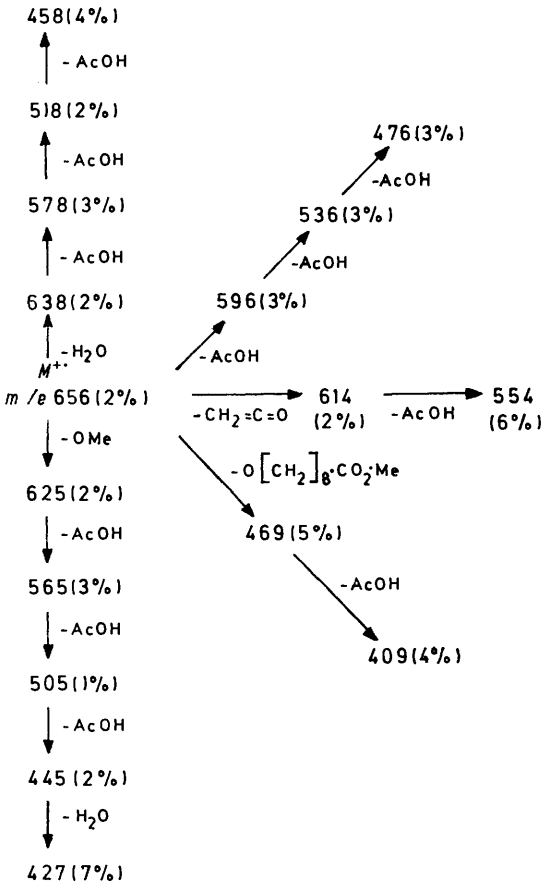
(X) (XI)

From the above discussion it follows that methyl pseudomonate B and pseudomonic acid B have structures (IIb) and (IIa), respectively.

The mass spectrum of the hydroxy-triacetate (IV) showed in the higher mass region the sequential loss of three acetic acid residues, either directly from $M^{+\cdot}$ (*m/e* 656), or following the initial loss of H_2O or OMe (Scheme 1). The fragment ions at *m/e* 270, 157, and 111 (Scheme 2) were also prominent fragments in the mass spectrum of methyl pseudomonate triacetate. The fragmentation depicted in route (a) followed by the sequential loss of acetic acid and/or water, in addition to the extra cleavage of the C(8)–C(9) bond generating *m/e* 485, adds further support to the proposed structure. The latter fragmentation was also seen in the mass spectrum of the hydroxy-methyl ketone triacetate (VIa), $M^{+\cdot}$ 444 (Scheme 3).

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage apparatus. U.v. spectra were measured for solutions in chloroform, i.r. spectra for solutions in carbon tetrachloride, and 100 MHz ^{1}H n.m.r. spectra for solutions in deuteriochloroform (tetramethylsilane as internal reference). The ^{13}C n.m.r. spectrum was recorded with a Varian XL-100 spectrometer, operating in the pulse Fourier transform mode. Mass spectra were recorded at 70 eV with an A.E.I. MS9 high resolution spectrometer. Optical rotations were measured



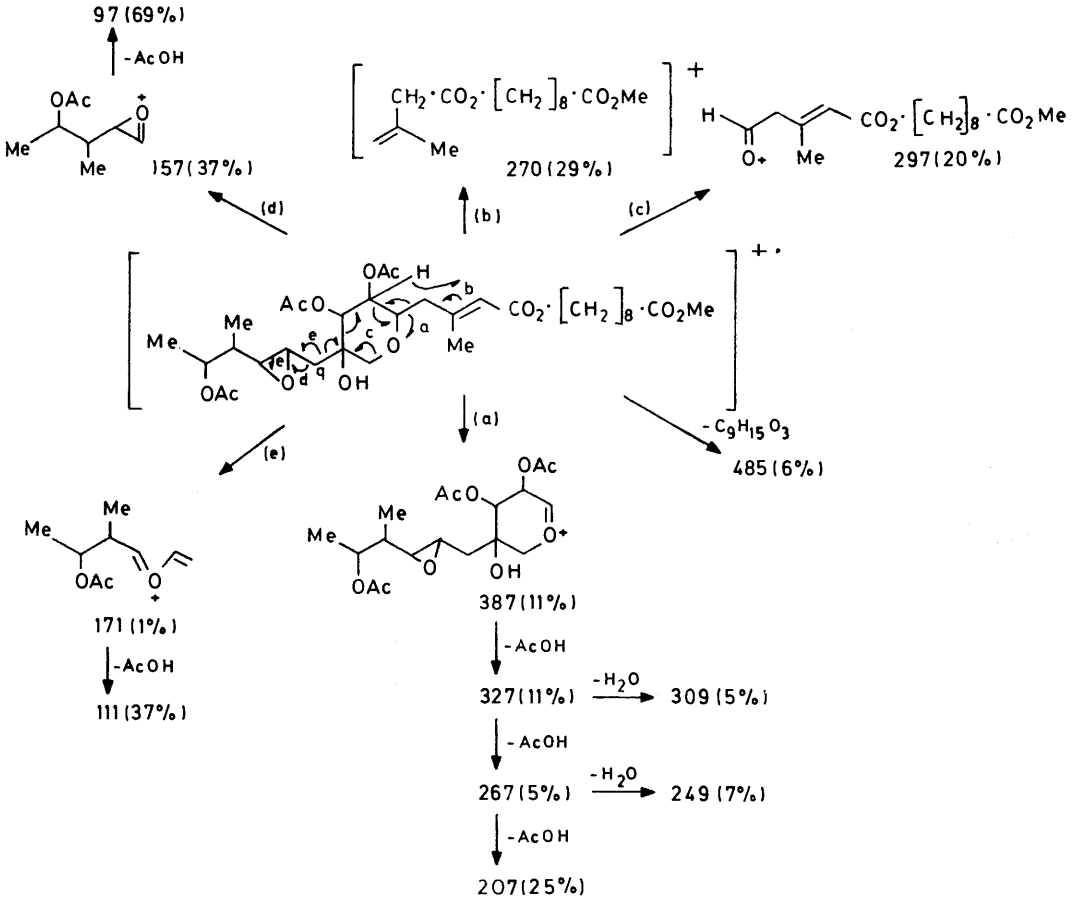
SCHEME 1

for solutions in chloroform at room temperature with a Perkin-Elmer 141 polarimeter. Evaporation refers to evaporation under diminished pressure. Thin-layer (t.l.c.) and preparative layer chromatography (p.l.c.) were performed on silica gel GF_{254} (Merck).

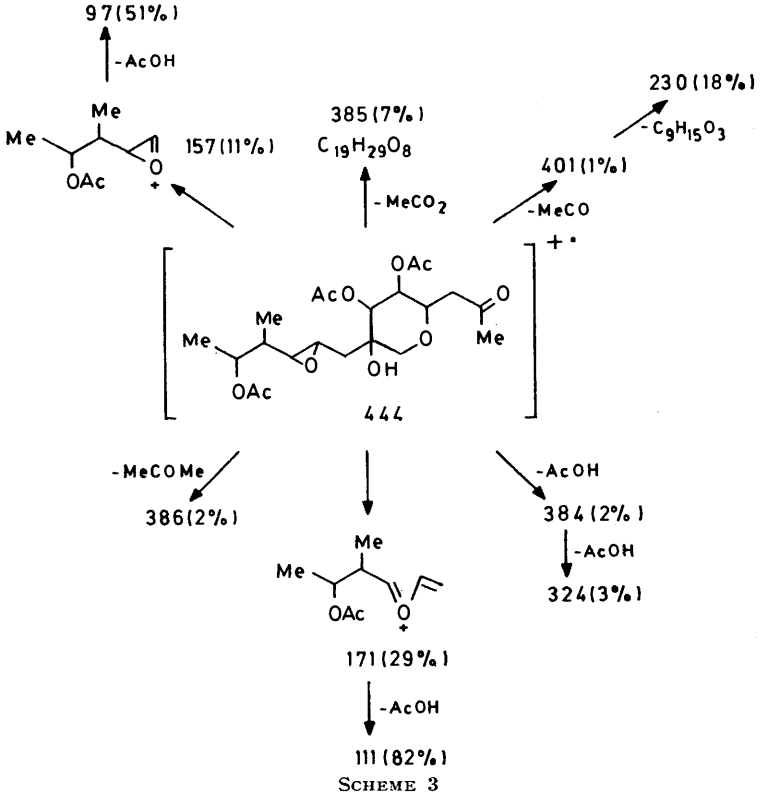
Methyl Pseudomonate B (IIb).—P.l.c. of the methylated (CH_2N_2), acidic antibiotic fraction (2.4 g) from a 3 000 l fermentation of *Pseudomonas fluorescens* [4] afforded oily *methyl pseudomonate B* (IIb) (238 mg), $[\alpha]_D$ 0° (*c* 2), $\nu_{max.}$ 3 650—3 100 (OH), 1 740infl, 1 720, 1 655, 1 225, and 1 150 cm^{-1}, $\lambda_{max.}$ 221 nm (ε 13 000), τ 9.12 (3 H, d, *J* 7 Hz), 8.78 (3 H, d, *J* 6.5 Hz), 8.70 (*ca.* 10 H, s), 7.83 (3 H, s), 6.55 (2 H, s), 6.40 (3 H, s, OMe), 5.98 (2 H, t, *J* 6.5 Hz), and 4.31br (1 H, s), *m/e* 530 ($M^{+\cdot}$), 512, 499, 494, 481, 476, 463, 428, 401, 383, 343, 325, 307, 270, 243, 255, 141, 115, 111, 97, 83, 71, 69, 67, and 45 (Found: C, 60.9; H, 8.5. $C_{27}H_{46}O_{10}$ requires C, 61.1; H, 8.7%).

The Hydroxy-triacetate (IV).—Methyl pseudomonate B (80 mg) was treated with acetic anhydride (0.5 ml) in

[7] G. S. Fonker and R. A. Johnson, 'Chemical Oxidations with Microorganisms,' Dekker, New York, 1971, p. 1.



SCHEME 2



SCHEME 3

pyridine (1 ml), overnight at ambient temperature. The mixture was poured into water and the product extracted into chloroform. The extract was dried and evaporated and the product purified by p.l.c. [propan-2-ol–chloroform (3 : 97)]. The band at R_F 0.5 afforded the oily hydroxy-triacetate (IV) (51 mg), $[\alpha]_D$ −1° (*c* 1.5), $\nu_{max.}$ 3 590 (OH), 1 740, 1 720, 1 650, 1 240, 1 150, 1 110, 1 040, and 940 cm^{-1}, $\lambda_{max.}$ 220 (ε 10 500), τ 9.05 (3 H, d, *J* 7 Hz), 8.75 (3 H, d, *J* 6.5 Hz), 8.82 (*ca.* 10 H, s), 8.05 and 7.98 (2 × 3 H, s, acetates), 7.87 (6 H, s, acetate plus vinylic Me), 7.40br (1 H, s) (OH, disappeared with D_2O), 7.25 (H^l, dd, *J* 7.5 and 2 Hz), 7.06 (H^k, finely split t, *J* 2 and 6 Hz), 6.48 (H^g and H^h, s), 6.24 (H^c, m), 6.00 (3 H, s, OMe), 5.36 (H^d, *J* 3 and 9 Hz), 5.06 (H^n, quint, *J* 5 and 6.5 Hz), 4.65 (H^e, d, *J* 3 Hz), and 4.37br (1 H, s); *m/e* 656 ($M^{+\cdot}$, $C_{33}H_{52}O_{13}$).

The Hydroxy-methyl Ketone Triacetate (VIa).—To the triacetate (IV) (50 mg) in pyridine (10 ml) was added osmium tetraoxide (100 mg), with stirring at room temperature. After 2 h the mixture was cooled to 4 °C and sodium hydrogen sulphite (400 mg) in water (10 ml) was added over 30 min. After a further 1½ h, the mixture was poured into water (50 ml) and the product extracted with ether containing 5% ethanol. The extract was dried and evaporated to give the diol (III), which was not characterised. To a solution of the diol in ethanol (30 ml) and water (5 ml) at ambient temperature was added a solution of sodium periodate in water (4 ml), with stirring. After 1 h the mixture was poured into water (200 ml) and extracted into ether. The extract was dried and evaporated leaving an oily residue which was eluted twice on p.l.c. with propan-2-ol–chloroform (1 : 99). The band at R_F 0.65 afforded 8-methoxycarbonyloctyl glyoxylate (V) (18 mg); semicarbazone, m.p. 162.5—164.5° [mixed m.p. with semicarbazone formed from (Ib) [4] showed no depression]. The band at R_F 0.35 afforded the oily *hydroxy methyl ketone triacetate* (VIa) (18 mg), $[\alpha]_D$ 0° (*c* 1), $\nu_{max.}$ 3 610 (OH), 1 745 (acetates), 1 725 (MeCO), 1 245, and 1 040 cm^{-1}, τ 9.04 (3 H, d, *J* 7 Hz), 8.75 (3 H, d, *J* 6.5 Hz), 8.48 (H^m, m), 8.21 (H^i, dd), 8.06 (1 H, dd), 8.06 and 7.98 (2 × 3 H, s, acetates), 7.87 (6 H, s, acetate plus MeCO), 7.56 (H^a, dd), 7.46 (H^b, dd), 7.25 (H^l, dd), 7.06 (H^k, seven lines), 6.46 (H^g and H^h, s), 5.91 (H^c, oct), 5.33 (H^d, dd), 5.06 (H^n, eight lines), and 4.64 (H^e, d), $J_{a,b}$ 14, $J_{a,c}$ 4.5, $J_{b,c}$ 7.5, $J_{c,d}$ 10, $J_{d,e}$ 3, $J_{i,j}$ 14, $J_{i,k}$ 7, $J_{j,k}$ 5, $J_{k,l}$ 2, $J_{l,m}$ 8.5, $J_{m,n}$ 5, $J_{Me,m}$ 7, $J_{Me,n}$ 6.5 Hz, *m/e* 444 ($M^{+\cdot}$, 1%, $C_{21}H_{32}O_{10}$), 401, 386, 385 (Found: 385.1868. $C_{19}H_{29}O_8$ requires 385.1862), 384, 366, 324, 230, 171, 170, 157, 129, 113, 111, 110, 97, 95, 69, 57, and 43.

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