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BACOGENIN-A_1: A NOVEL DAMMARANE TRITERPENE SAPOGENIN FROM *BACOPA MONNIERA**†

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Key Word Index—*Bacopa monniera*; Scrophulariaceae; Sapogenin; bacogenin-A_1; dammarene-type triterpene.

Abstract—The constitution of bacogenin-A_1, obtained from the acid hydrolysate of bacoside-A, has been established as 3,18-dihydroxy-20 → 25-epoxy-22(or 23)-methyl-24-nor-dammar-22-en-16-one. The treatment of di-*O*-acetylbacogenin-A_1 with RuO_4 led to a diol instead of the usual dioxo product.

INTRODUCTION

THE TWO crystalline saponins, bacoside-A, m.p. 250° and bacoside-B, m.p. 203° isolated earlier from *Bacopa monniera* Wettst. on acid hydrolysis, yielded glucose and arabinose and a mixture of four aglycones which were designated as bacogenins-A_1, m.p. 242°, R_f 0·15; A_2, m.p. 222°, R_f 0·19; A_3, m.p. 190°, R_f 0·34; and A_4, m.p. 170°, R_f 0·40 (TLC on silica gel G, C_6H_6 + 5% MeOH).[1]

Bacogenin-A_1, the most polar component in the aglycone mixture, was anticipated to be structurally closest, if not identical, to the genuine sapogenin. On the basis of earlier chemical data its structure was tentatively proposed as a trihydroxydammar-en-one.[1] The present paper deals with the detailed physico-chemical studies carried out subsequently which have led to the elucidation of the structure of bacogenin-A_1 as a dammarene having a rearranged side chain.

RESULTS AND DISCUSSIONS

Bacogenin-A_1, $C_{30}H_{48}O_4$ (M^+ 472) revealed the presence of OH (3410, cm^{-1}), C=O in a 5-membered ring (1730 cm^{-1}), *gem.* dimethyl (1375 cm^{-1}, (*d*), C=C–H (1660, 820 cm^{-1}), and ether (1080 cm^{-1}). Its PMR spectrum displayed signals for seven quaternary methyls (3H each, *s*, 0·783, 0·875, 0·958, 1·06, 1·26, 1·38, 1·53 ppm), $-C\underline{H}_2COC\underline{H}$ (3H, *bs*, 2·20 ppm), $-C\underline{H}-O-$(1H, *q*, *J* 9·5 and 5 Hz, 3·18 ppm), $-C\underline{H}_2-O-$(2H, *AB q*, J_{AB} 11 Hz centered at 3·95 ppm), $C\underline{H}_3-C=C-\underline{H}$ (3H, *d*, *J* 1·5 Hz, 1·63 ppm and 1H, *q*, *J* 1·5 Hz, 5·35 ppm). On spin decoupling, the quartet at 5·35 ppm collapsed to a singlet, thus confirming the assignments. Bacogenin-A_1 yielded a diacetate, m.p. 220, $C_{34}H_{52}O_6$ (PMR: two acetyl singlets

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† Presented at the 8*th International Symposium on the Chemistry of Natural Products*, New Delhi, India (1972).

[1] CHATTERJI, N. RASTOGI, R. P. and DHAR, M. L. (1965) *Indian J. Chem.* 3, 24.

at 2·01 and 2·05 ppm) demonstrating the presence of two acylable hydroxy groups. The PMR spectrum of bacogenin-A_1 was also recorded after addition of trichloroacetyl isocyanate (TAI),[2] which showed two broad singlets for –CONH–CO at 8·46 and 8·51 ppm confirming the presence of two hydroxy groups in the molecule.

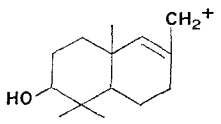
Bacogenin-A_1 formed a monooxime, m.p. 165°, $C_{30}H_{49}NO_4$, and a mono-2,4-dinitrophenylhydrazone, m.p. 110–112°, though in a low yield. The reduction of bacogenin A_1 with $LiAlH_4$ led to a trihydroxy derivative, m.p. 252°, $C_{30}H_{50}O_4$ (M^+ 474) and on Wolff-Kishner reduction desoxobacogenin-A_1, m.p. 215°, $C_{30}H_{50}O_3$ (M^+ 458) was obtained. Both derivatives lacked carbonyl absorption in the IR spectrum. The oxidation of bacogenin-A_1 with *tert.* butyl chromate followed by Wolff-Kishner reduction furnished a desoxydesoxo derivative, m.p. 185°, $C_{30}H_{50}O$ (M^+ 426), for which the hydroxyl and carbonyl absorptions were found to be absent in the IR spectrum. Thus, bacogenin-A_1 must be an unsaturated triterpenoid carrying 2 OH, a C=O and an inert O probably in the form of an ether (IR).

In order to define the basic skeleton, the $LiAlH_4$ product of bacogenin-A_1 was dehydrogenated with selenium and 6-hydroxy-1,2,5-trimethylnaphthalene (m.p. 140–142°) was identified[3] along with some other naphthalene derivatives in minor quantities which could not be identified. Bacogenin-A_1, therefore, most probably belongs to the dammarane group of tetracyclic triterpenoids.

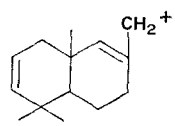
Functional Groups

The locations of the two hydroxy groups were established by the PMR and MS analysis of bacogenin-A_1 and its derivatives. In bacogenin-A_1 diacetate, the –CH–O quartet shifted downfield by more than 1 ppm and the $-CH_2-O$ protons moved downfield by 0·5 ppm indicating the presence of a secondary and a primary hydroxyl groups in the molecule. An axial–axial and axial–equatorial coupling of 9·5 and 5 Hz respectively of the –CH–OH proton was consistent with 3β (e) position of the hydroxyl in the dammarane skeleton. This inference was also supported by the fact that out of the seven quaternary methyls (in the PMR of bacogenin-A_1) those located at 0·783 and 0·875 ppm moved to 0·866 ppm on acetylation. This convergence of methyl signals on acetylation is frequently observed for 4α and 4β methyls in the triterpenoids having a 3β –OH group.[4–6]

The MS of bacogenin-A_1 displayed a molecular ion peak at *m/e* 472 and other prominent peaks at *m/e* 457 (M-15), *m/e* 439 (M-15-18), *m/e* 207 corresponding to I and an ion at *m/e* 189 (II) generated by the loss of water.



(I) *m/e* 207



(II) *m/e* 186

The formation of fragment ions I and II demonstrated that CH_2OH, C=O, Me –C=C–H groups and the inert oxygen were all present in the remaining part of the molecule. An

[2] TREHAN, I. R., MONDER, C. and BOSE, A. K. (1968) *Tetrahedron Letters* 67.
[3] FISCHER, F. G. and SEILER, N. (1959) *Ann.* **626,** 185.
[4] LEHN, J. M. and OURISSON, G. (1962) *Bull. Soc. Chim. Fr.* 1137.
[5] LEHN, J. M. (1962) *Bull. Soc. Chim. Fr.* 1832.
[6] ENTWISTLE, N. and PRATT, A. D. (1968) *Tetrahedron* **24,** 3949.

extremely intense peak at *m/e* 125 (base peak) has been assigned to the side chain (as in case of panaxadiol[7]) which, on further fragmentation, gave rise to *m/e* 107 by loss of H_2O (m* at *m/e* 91·4),[7,8] and *m/e* 110 by loss of a Me.

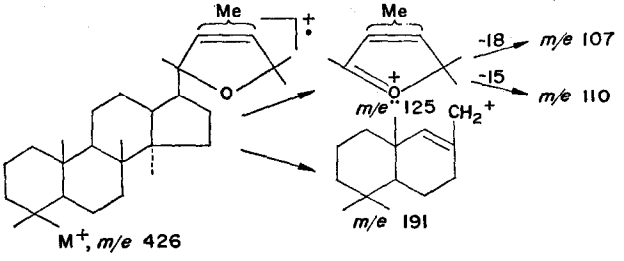
The base peak at *m/e* 125 also persisted in the MS of bacogenin-A_1 diacetate, indicating that the side chain did not contain the $-CH_2OAc$ group. Therefore, the primary hydroxyl group was fixed at C-18, the only position available in the molecule.

The keto group in a 5-membered ring, as indicated by the IR spectrum, could be placed at either C-15, C-16 and C-17 in ring D or in the side chain. The latter possibility was eliminated by the MS of bacogenin-A_1 derivatives devoid of keto groups, namely the $LiAlH_4$ product, desoxobacogenin-A_1 and desoxydesoxobacogenin-A_1. They displayed intense base peaks at *m/e* 125 demonstrating that the side chain remains intact in these derivatives. The position 17 in ring D is ubiquitously occupied by the side chain in the tetracyclic triterpenoids and, therefore, the location of keto group was narrowed down to position 15 or 16. The former position was untenable on the gound that an α-CH_2OH group would have been eliminated as HCHO by retroaldol condensation[8,9] during the reduction of bacogenin-A_1. Since this did not happen, the keto group must be placed at position 16. This position being somewhat hindered, would also account for the low yield of oxime and DNP derivatives, reported above. The positions of the side chain and the carbonyl group were further confirmed by the analysis of the PMR spectrum of bacogenin-A_1 in pyridine d_5, which showed (beside other signals) a 2H *AB* quarter (*J* 15 Hz) centered at 2·5 ppm and a 1H singlet at 2·6 ppm for $-CH_2-CO-CH-$ grouping.

Structure of the Side Chain

The side chain must accommodate the remaining components of the molecule namely Me–C=CH grouping, 3 quaternary methyls, and an ether linkage. In this respect desoxydesoxobacogenin-A_1 was of particular interest as it carries no skeletal functional groups but an intact side chain. Its MS showed a pattern (Scheme 1) similar to that of bacogenin-A_1 with peaks at *m/e* 426 (M^+), 411 (M-15), 393 (M-15-18), 191, 125 (base peak), 110 (125-15), 107 (125-18 *m** at 91·4).[7,8]

The elemental composition of the fragment ion *m/e* 125 was calculated as $C_8H_{13}O$ from the percentage abundances of the ions at *m/e* 126 (P + 1) and 127 (P + 2). The side chain of this composition having a double bond (1660, 820 cm^{-1}) should, therefore, bear the ethereal oxygen (1080 cm^{-1}) in the form of an oxide ring.



SCHEME 1. MS PRODUCTS FROM BACOGENIN-A_1.

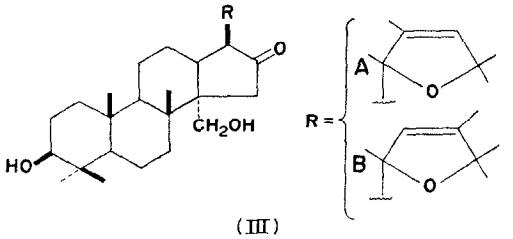
The PMR spectrum of desoxydesoxobacogenin-A_1 displayed a Me–C=C–H grouping (3H, *d*, *J* 1·5 Hz, 1·6 ppm and 1H, *q*, *J* 1·5 Hz, 5·21 ppm) and eight quaternary methyls,

[7] ELYAKOV, G. B., DZIZENKO, A. K. and ELKIN, YU. N. (1966) *Tetrahedron Letters* 141.
[8] AUDIER, H., BORY, S. and FÉTIZON, M. (1964) *Bull. Soc. Chim. Fr.* 1381.
[9] BARTON, D. H. R. and DE MAYO, P. (1954) *J. Chem. Soc.* 887.

five of which manifested the same magnetic environment as 5 skeletal methyls (4α, 4β, 8β, 10β and 14α) of 20ζ -hydroxy-dammar-24-ene (3H, *s* at 0·816, 0·90; 0·975 and 6H, *s*, at 0·858 ppm). The remaining 3 methyls were much deshielded (3H, *s* at 1·23 and 6H, *s* at 1·30 ppm) evincing their presence on carbons bearing oxygen with additional deshielding by the double bond, the only functional group present in the molecule. Further, there was no signal for protons situated on carbon bearing oxygen function. This indicated that the ether bridge was through fully substituted carbon atoms. The tertiary nature of the ether linkage was in conformity with its inertness towards the usual cleavage reagents such as BF_3–AC_2O, *p*-toluenesulphonic acid–AC_2O, H_2SO_4–HOAc, $LiAlH_4$, Na–NH_3, Li–NH_3. With BBr_3 an unseparable complex mixture with strong streaking on TLC was obtained.

The double bond in the side chain was also resistant to the usual splitting reagents namely alk. $KMnO_4$, O_3 and OSO_4. However, diacetylbacogenin-A_1 formed an epoxide, $C_{34}H_{52}O_7$ (M^+ 572, base peak *m/e* 141), m.p. 185°, on treatment with *m*-chloroperbenzoic acid. Its PMR spectrum showed singlets for a proton and a methyl group adjacent to an epoxide at 3·25 and 1·26 ppm respectively thereby confirming the relative positions of vinylic H and Me. These requisites could only be satisfied if the side chain has the structure A or B, which would also confer inertness on the ether linkage and the double bond. Regarding the stereochemistry at C-17, an α-configuration would be susceptible to epimerization on treatment with alkali because bacogenin-A_1 carries a carbonyl group at C-16. It was, however, recovered unchanged on alkali treatment showing that the side chain has a β-configuration as normally encountered in dammarane group of triterpenoids.

The structure for bacogenin-A_1 is, therefore, proposed as IIIA or IIIB. Because of the inertness of the double bond and the ethereal oxygen, it has not been possible to fix the position of the vinylic methyl group at C_{22} or C_{23}.



(III)

It is interesting that while attempting to cleave the double bond by treating diacetylbacogenin-A_1 with RuO_4, an unexpected hydroxylation took place leading to the formation of a diol, m.p. 138°, $C_{34}H_{54}O_8$ (M-60 at *m/e* 530). In its PMR spectrum the vinylic H and Me signals were replaced by singlets for C<u>H</u>–O at 3·73 ppm (shifted downfield by 1·36 ppm on acetylation) and a Me at 1·26 ppm. The PMR spectrum after addition of TAI showed two broad singlets at 8·60 and 9·00 ppm (CON<u>H</u>–CO–) confirming the presence of two hydroxy groups in the product. The diol could not be split by periodate probably due to the highly hindered nature of the glycol grouping. This, to our knowledge, appears to be the first example of a diol formation in reaction of RuO_4 with an olefin which is reported to give rise to dioxo compound.

EXPERIMENTAL

All m.ps were determined on Kofler block and are uncorrected. PMR spectra were recorded in $CDCl_3$ with TMS as internal standard. All the R_fs relate to TLC on silica gel plates.

Bacogenin-A_1. M.p. 242° (C_6H_6), $[\alpha]_D$ −70° (*c* 1%, $CHCl_3$). ν_{max}^{KBr}, 3410, 1730, 1660, 1450, 1375 (d), 1080, 820 cm^{-1}. PMR. ppm 0·783, 0·875, 0·958, 1·06, 1·26, 1·38, 1·53 (3H each *s*, 7 × Me), 1·63 (3H, *d*,

J 1·5 Hz, C=C–Me), 2·2 (3H, *bs*, CH_2–COCH), 3·18 (1H, *q*, *J* 9·5 and 5 Hz, CH–O–), 3·95 (2H, *AB q*, J_{AB} 11 Hz, CH_2–O–), 5·35 (1H, *q*, *J* 1·5 Hz C=C–H). PMR (pyridine-d_5): ppm 0·94, 1·02, 1·30, 1·47, 1·58 (3H each, *s*, 5 × Me), 1·16 (6H, *s*, 2 × Me), 1·79 (3H, *d*, *J* 1·5, C=C–Me), 2·5 (2H, *AB q*, *J* 15 Hz, CH_2–CO–), 2·60 (1H, *s*, CH–CO–), 3·39 (1H, *t*, *J* 8 Hz, CH–O–), 4·25 (2H, *AB q*, *J* 12 Hz, CH_2–O–), 5·33 (1H, *q*, *J* 1·5 Hz, C=C–H). MS: *m/e* 472 (M^+), 457 (M-15), 439 (M-15-18), 207, 189, 125 (base peak), 110, 107 (125-18, *m** at 91·4). (Found: C, 75·74; H, 10·8. $C_{30}H_{48}O_4$ requires: C, 76·27; H, 10·16%.)

Di-O-acetylbacogenin-A_1. M.p. 220°, $\nu_{max}^{CHCl_3}$, 1750, 1730, 1440, 1360, 1250, 1025, 970, 820 cm^{-1}. PMR. ppm 0·807 (6H, *s*, 2 × Me), 0·91, 1·1, 1·28, 1·38, 1·53 (3H, *s*, 5 × Me), 1·633 (3H, *d*, *J* 1·5 Hz, C=C–Me), 2·01, 2·05 (3H each, *s*, 2 × OCO Me), 2·13 (1H, *s*, CO–CH), 2·2 (2H, *s*, $COCH_2$), 4·45 (2H, *AB q*, J_{AB} 13 Hz, CH_2–O–CO–), 4·53 (1H, *m*, CH–O–CO–), 5·41 (1H, *q*, *J* 1·5 Hz, C=C–H).

$LiAlH_4$ *reduction of bacogenin-*A_1. The reduction product[1] was obtained as colourless needles from EtOAc, m.p. 252°. PMR. ppm 0·78, 0·866, 0·966, 1·06, 1·28, 1·33, 1·48 (3H each, *s*, 7 × Me), 1·61 (3H, *d*, *J* 1·5 Hz, C=C–Me), 3·21 (1H, *m*, CH–O–), 3·78 (2H, *AB q*, *J* 12 Hz, CH_2–O–), 4·38 (1H, *m*, CH–O–), 5·25 (1H, *q*, *J* 1·5 Hz, C=C–H).

Selenium dehydrogenation. Bacogenin-A_1 (1 g) was reduced with $LiAlH_4$ (1 g) in Et_2O. The resulting product was mixed with Se powder (2·5 g) and heated at 320° for 20 hr in N_2. The reaction mixture was exhaustively extracted with Et_2O and the ethereal solution was washed with NaOH solution (10%), aq. HCl (10%) and finally with H_2O. The solution was then evaporated and the residue (0·67 g) was chromatographed over Al_2O_3 (20 g). The Et_2O eluate (300 mg) was rechromatographed over alumina column and the major component (a) was further purified by preparative TLC in light petrol. Three UV-fluorescent zones were isolated in the usual manner. The product (a_3), 15 mg, was sublimed at 175°/1–2 mm and the sublimate crystallized from $CHCl_3$–light petrol, m.p. 140–142°, λ_{max} 223 (log ε 4·58), 275 (log ε 4·27), 282 (log ε 4·29), 291 (log ε 4·22), 326 (log ε 3·78) nm identified as 6-hydroxy-1,2,5-trimethylnaphthalene. The product (a_2), 18 mg, amorphous powder from MeOH, m.p. 156–160°, λ_{max} 229, 273, 308, 327 nm as well as other minor components could not be characterized.

Desoxobacogenin-A_1. Bacogenin-A_1 (150 mg) was suspended in diethylene glycol (15 ml) and then Na (400 mg) and N_2H_4–H_2O (99–100%, 2 ml) were added. The reaction mixture was heated at 110° for 3 hr, refluxed at 190° for 3 hr, cooled, diluted with H_2O (30 ml) and extracted with C_6H_6 (3 × 15 ml). The organic layer was evaporated and the residue was chromatographed over neutral Al_2O_3 (act. 3, 6 g). The C_6H_6–$CHCl_3$ (3:1) eluate (75 mg) crystallized from C_6H_6-cyclohexane, m.p. 215° (60 mg). ν_{max}^{KBr} 3400, 2900, 2850, 1198, 1010, 970, 820. PMR. ppm 0·73, 0·866, 1·28 (3H each, *s*, 3 × Me), 1·00, 1·33 (6H each, *s*, 4 × Me), 1·61 (3H, *d*, *J* 1·5 Hz, C=C–Me), 3·25 (1H, *m*, CH–O–), 3·80 (2H, *AB q*, J_{AB} 11 Hz, CH_2–O–), 5·30 (1H, *q*, *J* 1·5 Hz, C=C–H). MS. *m/e* 458 (M^+), 443 (M-15), 425 (M-15-18), 409, 125 (base peak), 107 (125-18). (Found: C, 79·1; H, 10·60. $C_{30}H_{50}O_3$ requires: C, 78·6; H, 10·91%.)

Desoxydesoxobacogenin A_1. To a solution of bacogenin-A_1 (100 mg) in $CHCl_3$–*t*-butanol (4:1) was added *t*-butyl chromate in CCl_4 (1%, 1·5 ml) over a period of 2 hr. The reaction mixture was allowed to stand for 1 hr, then worked up. The resultant product was dissolved in $CHCL_3$ and filtered through neutral Al_2O_3. The filtrate was evaporated to a residue (85 mg) which showed on TLC one major product (R_f, 0·8 in C_6H_6–cyclohexane, 1:1) and some minor impurities. The major component could not be crystallized and was found to be so unstable that it changed into a number of products within 2–3 days even on storing in a refrigerator. The residue as such was, therefore, refluxed with N_2H_4–H_2O (99–100%) and Na in diethylene glycol as described earlier. The reaction product (68 mg) was chromatographed over neutral Al_2O_3 (act. 3, 4 g). The light petrol. eluate (35 mg) on crystallization from $CHCl_3$–MeOH yielded fine colourless needles (25 mg), m.p. 183–185°, ν_{max}^{KBr} 2950, 2825, 1460, 1370, 1180, 1080, 970, 840, 820 cm^{-1}. PMR. ppm 0·81, 0·95, 1·23 (3H each, *s*, 3 × Me), 0·858, 1·30 (6H each, *s*, 4 × Me), 1·6 (3H, *d*, *J* 1·5 Hz, C=C–Me), 5·21 (1H, *d*, *J* 1·5 Hz, C=C–H). MS. m/e 426 (M^+), 411 (M-15), 393 (M-15-18), 125 (base peak), 107 (125-18, *m** at 91·4).

Di-O-acetylbacogenin-A_1*-epoxide.* A solution of *m*-chloroperbenzoic acid (200 mg) in dry $CHCl_3$ (10 ml) was added to a solution of di-*O*-acetylbacogenin-A_1 (200 mg) in $CHCl_3$ (10 ml) and left for 8 days at ambient temp. Excess of the reagent was destroyed by adding 10% sodium metabisulphite. The solution was then washed free of acid, dried and evaporated. The crude product gave fine colourless needles from EtOH, m.p. 185°, 185 mg, R_f 0·75 in C_6H_6 4% MeOH. ν_{max}^{KBr} 1750, 1730, 1250, 1035, 980, 850, 820 cm^{-1}. PMR. ppm 0·88 (6H, *s*, 2 × Me), 0·95, 1·13 (3H each, *s*, Me), 1·26 (6H, *s*, 2 × Me), 1·45, 1·53 (3H each, *s*, Me), 2·05, 2·1 (3H each, *s*, OCO Me), 2·2 (2H, *s*, CH_2–CO), 2·3 (1H, *s*, –CH–CO), 3·25 (1H, *s*, –CH–O), 4·53 (2H, *AB q*, *J* 12 Hz, CH_2O–CO), 4·61 (1H, *m*, CH–O). MS. *m/e* 572 (M^+), 554 (M-18), 539, 512 (M-60), 499 (M-73), 189, 141 (base peak), 99 (141–142, *m** at 69·5). (Found: C, 71·75; H, 8·78. $C_{34}H_{52}O_7$ requires: C, 71·32; H, 9·09%.)

RuO_4 *oxidation of di-O-acetylbacogenin-*A_1. Di-*O*-acetylbacogenin-A_1 (425 mg) in CCl_4 (10 ml) was cooled in ice and a solution of RuO_4 in CCl_4 (*ca.* 3%, 24 ml) was added dropwise with stirring. The mixture was stirred for 2 hr at 0° and for 2 hr at room temp. The excess of the reagent was decomposed with MeOH (1·5 ml) and the precipitated RuO_2 was filtered. The filtrate on evaporation gave a residue (450 mg) which

[10] POLONSKY, J. (1951) *Compt. Rend.* **233**, 93, 671.

showed one major component on TLC (R_f 0·18 in C_6H_6–MeOH 4%) along with some starting material. On its chromatography over silica gel (15 g), the $CHCl_3$ eluate yielded a pure product which crystallized from C_6H_6–light petrol., m.p. 138°, 180 mg. ν_{max}^{KBr} 3400, 2900, 2825, 1730 1450, 1375, 1245, 1075, 1025, 980, 955. PMR. ppm 0·883 (6H, *s*, 2 × Me), 0·91 (3H, *s*, Me), 1·06 (3H, *s*, Me), 1·26 (6H, *s*, 2 × Me), 1·33 (3H, *s*, Me), 1·4 (3H, *s*, Me), 2.1 (6H, *s*, 2 × –OCO Me), 3·73 (1H, *s*, CH–O–), 4·38 (2H, *d*, *J* 2 Hz, CH_2–O–CO–), 4·56 (1H, *m*, CH–O–CO–). MS. (M^+ not visible) *m/e* 530 (M-60), 519 (M-71), 502, 475, 470 (M-60-60), 459, 433, 415, 399, 373, 355, 329, 313, 189, 98. (Found: C, 69·50; H, 9·46. $C_{34}H_{54}O_8$ requires: C, 69·15; H, 9·15%.)

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