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STRUCTURAL AND STEREOCHEMICAL STUDIES ON MARINE NORTERPENE CYCLIC PEROXIDES

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Abstract - Isolation of a new norditerpene cyclic peroxide (12) and three new norsesterterpene cyclic peroxides (1, 2, 26) has permitted a detailed study of the stereostructures of this unusual class of marine natural product A procedure for assigning relative and absolute stereochemistry about the peroxy moiety is outlined An absolute stereostructure for muqubilin (11) and a revision of the previously assigned absolute stereochemistry of the sigmosceptrellins (5-10) is proposed, the latter based on the Horeau procedure

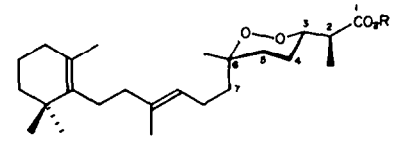
Despite the novelty of the peroxide moiety a number of stable cyclic peroxides have been isolated from marine sponges,[1-6] many of which have been reported to exhibit significant antibiotic activity In several cases the structural studies have been limited to gross structures leaving absolute[1] and even relative[4,5,6] stereochemistry unassigned In view of the potential to explore structure-activity relationships within this class of compounds it seemed worthwhile extending our investigations of new examples of this structural class to include a more general stereostructural examination In this paper we report a new norditerpene (12) and three new norsesterterpene (1,2, 26) peroxides as well as describing a general procedure for assigning stereochemistry to cyclic peroxides of this class

RESULTS AND DISCUSSION

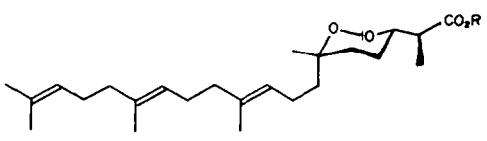
The ethanol extract of an unidentified sponge,[7] Z4967, collected from a depth of 25m off Durras, New South Wales, was found to show significant growth inhibitory activity against the gram positive bacteria Bacillus subtilis and the 'yeast' Saccharomyces cerevisae Trituration of the concentrated extract with dichloromethane yielded an antimicrobially active lipid soluble fraction Fractionation by rapid elution through silica resulted in the isolation of an interesting inactive compound (12) and an active two component mixture (1 + 2) Methylation of the latter with diazomethane yielded two inactive methyl esters 3 and 4 which were readily resolved on silver nitrate impregnated silica

(a) Structural Studies

The methyl esters 3 and 4 are colourless stable oils having a number of spectroscopic features in common Each displays a weak molecular ion at m/z 406 ($C_{25}H_{42}O_4$) in its respective mass spectrum, with significant fragment ions at m/z 388 (M^+-H_2O), 375 (M^+-OCH_3) and 319 ($M^+-CH_3CHCO_2CH_3$) ^1H and ^{13}C NMR resonances attributable to the methyl ester moiety were readily recognisable (δ 3 69 (s), ppm 174 4 (s), 51 8 (q)) The presence of only two additional



1 R=H
3 R-CH$_3$



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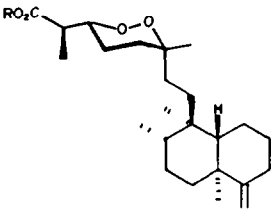
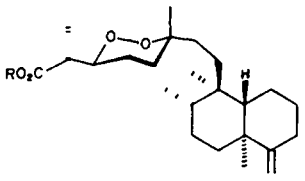
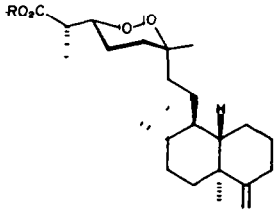
4 R=CH$_3$



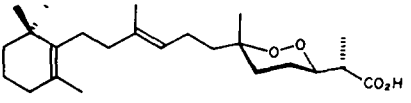
5 R=H
8 R-CH$_3$



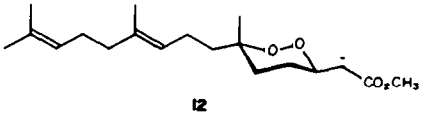
6 R-H
9 R-CH$_3$



7 R=H
10 R CH$_3$



MUQUBILIN
11



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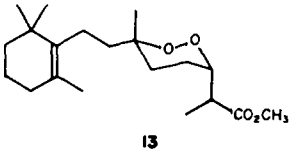


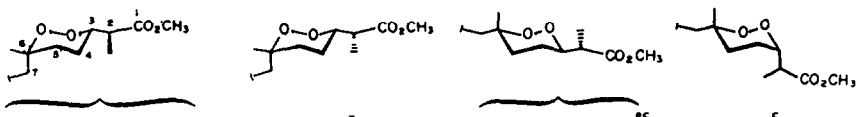
13

oxygen bearing carbons (3 81 3 (d), 79 9 (s), 4 81 2 (d), 79 8 (s)), combined with the absence of exchangeable resonances in the ^{1}H NMR spectra and lack of hydroxyl absorption in the IR spectra, required that the remaining two oxygen functionality in 3 and 4 be a cyclic peroxide A comparison (Table 1) of the spectral data for 3 and 4 with that of the recently reported sigmosceptrellins A, B and C (5, 6 and 7)[2,3] and their methyl esters (8, 9 and 10) confirmed that 3 and 4 possessed the same cyclic peroxide functionality

Further examination of the ^{1}H NMR data for 3 revealed resonances for two olefinic methyls (δ 1 61, 1 65, 2s), two equivalent tertiary methyls (1 00, s) and an olefinic proton (5 17, t) The ^{13}C NMR spectrum confirmed the presence of a trisubstituted double bond (137 1 (s), 123 5 (d)) with a trans configuration, as determined by the upfield shift of the associated olefinic methyl (15 9 ppm),[10] and a tetrasubstituted double bond (126 9 (s), 136 2 (s)), requiring a monocarbocyclic skeleton for 3 Complete assignment of the remaining carbon resonances by correlation with model compounds[4,5] (e g muqubilin 11, a geometric isomer of 1), together with a characteristic base peak in the mass spectrum at m/z 137, established the gross structure for 3

Table 1†

Selected ^1H and ^{13}C NMR ($CDCl_3$) shifts for Marine Norterpene Peroxides



	3	4	8	27	10[3]	12	9	11*[5]	13[5]
1	174 4	174 3	174 2	174 1	174 2	174 1	174 1	180 4	174 1
2	42 6	42 5	42 6	42 5	43 0	42 9	42 9	43 0	42 7
3	81 3	81 2	81 1	81 0	80 9	81 3	81 3	81 0	81 1
4	22 6	22 5	21 8	22 5	21 9	23 4	23 5	23 5	22 2
5	32 4	32 4	32 5	32 4	32 3	31 9	31 9	32 0	32 8
6	79 9	79 8	80 2	80 1	80 2	80 0	80 3	80 2	79 9
7	34 8	34 2				39 6		39 7	34 9
OCH_3	51 8	51 7	51 8	51 8	51 8	51 7	51 8		
2-CH_3	12 8	12 7	12 4	12 4	13 2	13 5	13 5	13 2	12 5
6-CH_3	23 9	23 8	24 0	24 0	23 5	20 5	20 9	20 8	23 6
2-H	2 57	2 56	2 56	2 56	2 59	2 65	2 65	2 54	2 58
3-H	4 24	4 23	4 23	4 23	4 15	4 12	4 11	4 06	4 25
O-CH_3	3 69	3 69	3 69	3 69	3 71	3 70	3 70	-	3 70
2-CH_3	1 14	1 14	1 13	1 13	1 22	1 23	1 24	1 24	1 13
6-CH_3	1 13	1 12	1 06	1 06	1 09	1 30	1 23	1 18	1 15

* data reported on the acid

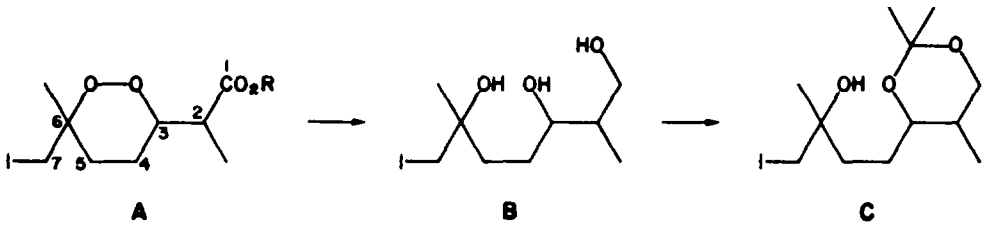
† structural headings imply relative stereochemistry only

Unlike that of 3, the ^1H NMR spectrum of 4 exhibited resonances for four olefinic methyls (δ 1 66, 1 66, 1 66, 1 74, 2S) and three olefinic protons (5 23, m) requiring an acyclic carbon skeleton The ^{13}C NMR spectrum revealed three tri-substituted double bonds (131 1 (s), 124 3 (d), 134 7 (s), 124 0 (d), 135 2 (s), 124 1 (d)) two of which were trans substituted [10] Comparison with model compounds[10] readily identified this portion of the molecule as a farnesyl unit Consistent with this assignment is the observation of a base peak at m/z 69 in the mass spectrum of 4

The mass spectrum of the inactive less polar component (12) contained a molecular ion at m/z 338 ($C_{20}H_{34}O_4$) with fragment ions at m/z 323 (M^+-CH_3), 320 (M^+-H_2O) and 251 ($M^+-CH_3CHCO_2CH_3$), and a base peak of m/z 69 The ^1H NMR spectrum of 12 showed resonances for a methyl ester (δ 3 70, s) a secondary methyl (1 26, d), a tertiary methyl (1 30, s), three olefinic methyls (1 60, 1 60, 1 67, 3S), two olefinic protons (5 09, t) and an oxymethine proton (4 12, ddd) A multiplet (dq, J=8 0, 8 0 Hz, δ 2 65) characteristic of the C2 alkylmethine proton in cyclic peroxides such as the sigmosceptrellins[3] was also present Comparison of the carbon resonances in the ^{13}C NMR spectrum of 12 with those attributed to the peroxy moiety in the methyl ester of sigmosceptrellin-B (9)[3] confirmed the presence of this functionality in 12 (see Table 1) The remaining carbon resonances included two trisubstituted double bonds, (131 2 (s), 124 2 (d) (135 3 (s), 123 8 (d), olefinic methyl 15 9 (q)), which could be assigned[10] to a trans geranyl unit

(b) Relative Stereochemistry

Although several marine natural products have been reported[2-6] incorporating the cyclic peroxide moiety **A** no convenient approach has been described for determining the relative stereochemistry about the three chiral centres C2, C3 and C6 (Scheme 1) The first natural product reported to contain this functionality was muqubilin[4] (**11**) isolated from a Red Sea sponge, Prianos sp In this initial report no attempt was made to secure the stereostructure of **11** nor was any mention made of its optical activity A later report[6] on the antibiotic activity of **11** (referred to as prianicin-A) attributed to it without elaboration the stereostructure of sigmosceptrellin-A (**5**) which had subsequently been isolated and subjected to X-ray analysis [2] More recently **11** was re-isolated from an unidentified sponge collected from Tonga[5] and the relative stereochemistry about C3 and C6 determined by NMR spectroscopy This report also described the norditerpene methyl nuapapuanoate (**13**) and assigned to it the partial stereostructure shown No comment was made in either case on the relative stereochemistry about C2, nor on the absolute stereochemistry The X-ray analysis[2] of **5** was the first instance where the relative stereochemistry about the three chiral centres C2, C3 and C6 in a cyclic peroxy functionality such as **A** had been unambiguously established Chemical interrelation[3] of **6** and **7** with **5** secured their relative stereochemistries



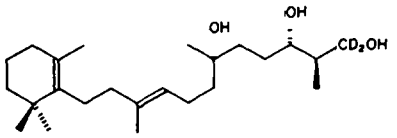
SCHEME 1

Examination of the ^{1}H and ^{13}C NMR data pertaining to the peroxy moiety in **3**, **4**, **8**, **9**, **10**, **11** and **12** (Table 1) indicates a number of features correlating with specific stereochemical configurations For example the ^{13}C NMR shifts associated with the C6 tertiary methyl in **3**, **4**, **8** and **10** (23 5 to 24 0 ppm) are significantly further downfield than the corresponding shifts in **12**, **9** and **11** (20 5 to 20 9 ppm) It is known[10] that an equatorial methyl on a six membered ring in a chair conformation resonates downfield of axially oriented analogues and that six membered cyclic peroxides exist in stable chair conformations [11] This effect can also be observed in the chemical shifts for C7 in **3** and **4** (34 2 to 34 8 ppm) vs **11** and **12** (39 5 to 39 7 ppm) Thus the relative stereochemistry about C6 in the peroxy functionality can readily be established by examining the ^{13}C NMR shifts for C7 and the C6 tertiary methyl Assignments made in this manner are in agreement with those previously determined[2,3,5] for **8**, **9**, **10** and **11** A contradiction exists in the case for **13** in which an axial orientation has been assigned to the C6 tertiary methyl at 23 6 ppm (equatorial C7 at 34 9 ppm) [5] In light of the trends displayed in Table 1 it would appear more reasonable to reassign the C6 methyl in **13** as equatorial

Interpretation of the ^{1}H NMR spin system for the C3 oxymethine proton provides evidence for the relative stereochemistry about this centre In **3**, **4**, **8**, **9**, **10** and **12** this system appears as a doublet of doublet of doublets with $J_{2,3}$ = 7-8 Hz, $J_{3,4e}$ = 3-4 Hz and $J_{3,4a}$ = 7-8 Hz The large $J_{3,4a}$ is indicative of an axial-axial coupling and therefore confirms the axial orientation of the C3 oxymethine proton In **11** this spin system was reported[5] to be unresolved but $J_{3,4a} + J_{3,4e}$ was quoted as 12 Hz, supporting an axial C3 oxymethine proton, while for **13** a $J_{3,4a} + J_{3,4e}$ of 8 Hz was taken as evidence[5] for an equatorial orientation

Of the three chiral centres in the cyclic peroxide moiety **A** the stereochemistry at C2 has proved the most elusive to determine X-ray analysis permitted assignment in the case of the sigmosceptrellins (**5-10**)[2,3] but for muqubilin (**11**) and methyl nuapapuanoate (**13**) the stereochemistry remained undefined [4-6] One approach to assigning the stereochemistry about C2 was to constrain this centre into a fixed and definable conformation relative to C3 The transformation outlined in Scheme 1 from the cyclic peroxide **A** via the triol **B** to the dioxan **C** achieves this Conformational studies[12-14] on dioxans such as **C** confirm that they adopt regular chair conformations in which the more bulky C3-substituent (numbering as for **A**) assumes an equatorial orientation Thus, depending on the relative stereochemistry about C2 in **A** the C2 secondary methyl in **C** will adopt either an axial or equatorial orientation, while the C3 oxymethine proton will be axial Assignment of the orientation of the C2 secondary methyl is simplified by the observation[14] that axial methyls in this position on dioxans such as **C** have ^{1}H NMR resonances downfield (~δ 1 1) of the corresponding equatorial analogues (~ 0 7) In addition analysis of the C3 oxymethine spin system in **C** can be used to assign stereochemistry To facilitate measurement of this multiplet the overlapping C1 oxymethylene protons were exchanged by carrying out the reduction with lithium aluminium deuteride Following this procedure **3**, **4** and **12** were reduced to the deuterated triols **14**, **15** and **16** which were subsequently protected as the isopropylidenes **17**, **18** and **19** ^{1}H NMR shifts for the C2 secondary methyl in **17** and **18** (δ 0 76) together with a $J_{2,3}$ of 10 1 Hz required an equatorial configuration In contrast the ^{1}H NMR shift for the secondary methyl in **19** (δ 1 07) and a $J_{2,3}$ of 5 1 Hz pointed to an axial orientation Verification of this approach to the relative stereochemistry about C2 was obtained by reduction of an authentic sample of the methyl ester of sigmosceptrellin-A (**8**) to give **20** followed by derivatisation to **21** As expected **21** possessed an equatorial C2 secondary methyl (δ 0 76, $J_{2,3}$ ~10 Hz)

Reexamination of the ^{1}H NMR shifts for the C2 secondary methyl in **3**, **4**, **8**, **9**, **10** and **12** revealed an interesting correlation In those cases where C2 and C3 are in an *erythro* configuration (*R*,*R* or *S*,*S*) as in **3**, **4** and **8**, the ^{1}H NMR shift for the C2 methyl is upfield (δ 1 13 to 1 14) while in those examples where they are *threo* (*R*,*S* or *S*,*R*) as in **9**, **10** and **12**, this methyl resonates downfield (1 24 to 1 26) (Table 1) A similar observation has recently been reported between the diastereomers of methyl nonactate [15] Thus it is possible to predict the relative stereochemistry about C2 and C3 from the ^{1}H NMR shift of the C2 secondary methyl Application of this approach to muqubilin (**11**) which exhibits a secondary methyl shift of δ 1 23 suggests a *threo* relative stereochemistry about C2 and C3 While this correlation seems to hold independently of the stereochemistry about C6 all examples considered possessed an axial C3 oxymethine proton For this reason it is inappropriate to extend this technique to assigning the stereochemistry about C2 in compounds such as methyl nuapapuanoate (**13**)[5] where the C3 proton is equatorial



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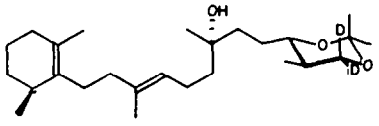

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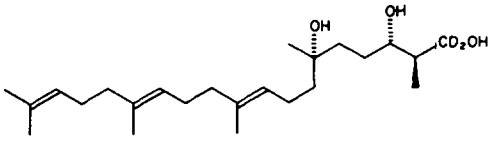

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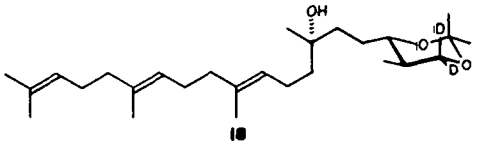

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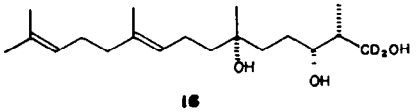

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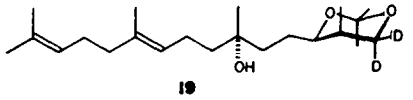

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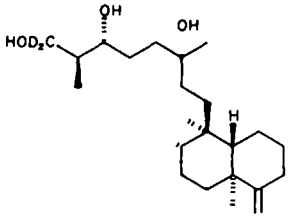

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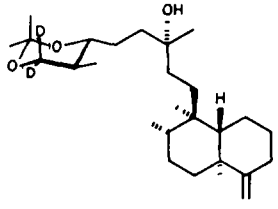

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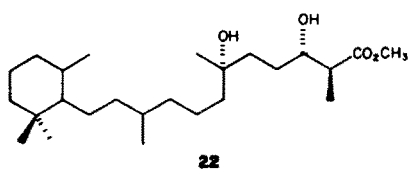
(c) Absolute Stereochemistry

All the peroxides discussed are optically active[16] and therefore are presumably the result of enzyme mediated biosynthesis The absolute stereochemistry at C2, C3 and C6 (numbering as in A) in 5 (2S, 3S, 6S), 6 (2S, 3R, 6S) and 7 (2R, 3S, 6S) have been reported, based on measurements on a a cyclic ketone derived from 6 [3] Such an approach was not appropriate for determining the stereochemistry about these centres in 3, 4 and 12 Instead these compounds were hydrogenated to the saturated diol esters 22, 23 and 24 and an attempt made to determine the absolute stereochemistry about the secondary hydroxyl by application of Horeau's procedure[18] of asymmetric esterification The results of these analyses are shown in Table 2 and suggest that 3 and 4 possess the same absolute stereochemistry about C2, C3 and C6 as 8 (2S, 3S, 6S) while 12 is the same as 9 (2S, 3R, 6S) However, when an authentic sample of 9 was treated in a similar manner a contradictory result was obtained The absolute stereochemistry for 9 determined by application of Horeau's procedure on its hydrogenation product 25 was opposite (2R, 3S, 6R) to that previously reported by interpretation of CD measurements [3] Although insufficient authentic 8 was available to carry out a similar comparison a supply of the enantiomer 27 was available A second sponge, Z4969, collected at the same locality as that previously described contained the norsesterterpene peroxide 26 as its major secondary metabolite To facilitate purification the crude ethanol extract was methylated with diazomethane and the methyl ester 27 isolated after elution through silica Compound 27



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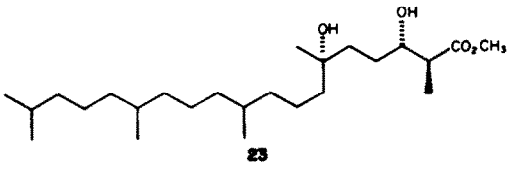

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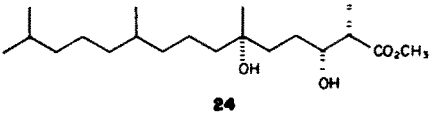

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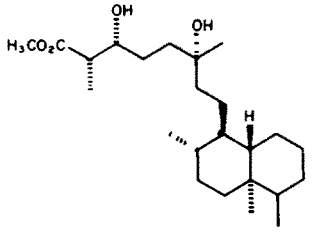

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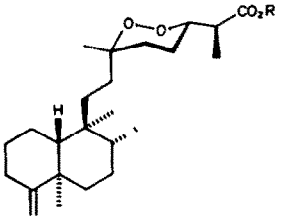

26 R = H
27 R - CH_3

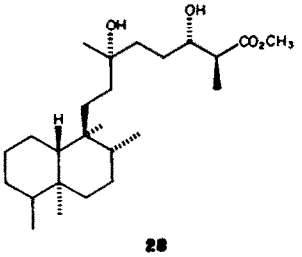

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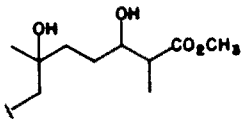
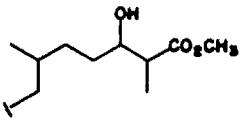
Table 2

Results of Horeau analyses on Secondary Alcohols derived from Marine Norterpene Peroxides

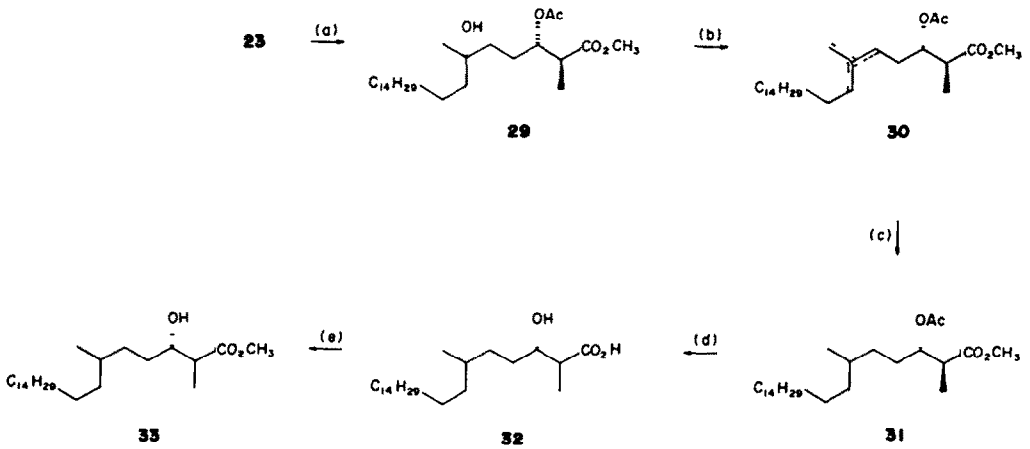




Derived Secondary Alcohol	22	23	24	25	28	33
% Esterification	100	100	100	100	100	100
$[\alpha]_D^{25}$ of partially resolved α-phenylbutyric acid	-9 4	-3 0	+3 7	-4 5	-3 0	-3 0
Optical Yield	29 4	9 3	11 7	15 3	9.3	9 3
Implied Stereochemistry	S	S	R	S	S	S
Norterpene Peroxide	3	4	12	9	27	4

exhibited spectroscopic characteristics identical to those of 8 but possessed an $[\alpha]_D$ opposite in sign, -57 1, and an ORD curve opposite to that of authentic 8, thus establishing 27 as the enantiomer of 8. Application of the Horeau procedure to 28, the hydrogenation product of 27, returned a C3 stereochemistry (S) opposite to that expected (R) by correlation with its enantiomer 8. Despite this apparent contradiction it is possible to establish from the Horeau analyses that the absolute stereochemistries about C2, C3 and C6 in 3, 4, 12 and 27 are opposite to those previously assigned to sigmosceptrellins of the same relative stereochemistry

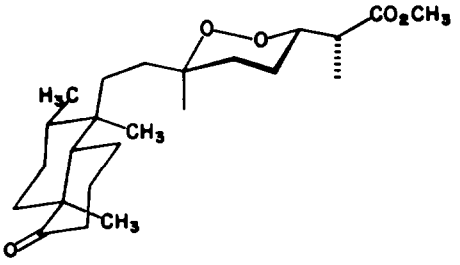
A possible explanation proposed for the lack of agreement between the results of the asymmetric esterification technique and assignments based on CD measurements was the influence of the C6 tertiary hydroxyl [19] If hydrogen bonding occured between the C2 and C6 hydroxyls this could influence the resolution process, using the Horeau method To examine this possibility a sample of **4** was treated as shown in Scheme 2 to remove the C6 hydroxyl Thus the diol ester **23** was acetylated to **29** which was dehydrated to a mixture of double bond isomers **30**. Hydrogenation to **31** followed by hydrolysis[20] yielded the hydroxy acid **32** which was methylated to the desired hydroxy ester **33**.



SCHEME 2

(a) pyridine/acetic anhydride, (b) pTsOH/Δ/benzene
(d) 10% Pd/C, H_2, (d) K_2CO_3/MeOH, (e) CH_2N_2

Asymmetric esterification of **33** with a two fold excess of racemic α-phenylbutyric anhydride resulted in recovery of a preponderance of (-)α-phenylbutyric acid correlating[18] with an S stereochemistry about C3 This was in agreement with that previously established from the diol ester **23** thus ruling out the above argument (Table 2). An alternative explanation is that the stereochemical assignment based on CD observations[3] is incorrect. The contribution of an axial α-methyl to the Cotton effect observed for steroidal ketones and cyclohexanone model compounds is known to be large [21] However, a CD prediction for 9(R)-methyl-trans-decal-1-one is reported[22] as 'probably positive', despite an expectedly strong positive axial α-methyl contribution, due to the negative influence of the remote C5 and C6 carbons. Furthermore, reference[22] to Δ-homoandrostane-3β-ol-17a-one and cholestan-1-one suggests that remote non-vanishing groups can override the more obvious α contribution. Such an explanation could account for the positive Cotton effect at 296 nm observed for[3] the 4-keto derivative **34** of sigmosceptrellin-B methyl ester. Ideally the question of absolute stereochemistry would best be resolved by application of an unambiguous degradative or synthetic approach. In the absence of such confirmation and in view of the observations above we favour reassignment of the absolute stereochemistry for the sigmosceptrellins (**5-10**) and corresponding assignment of stereochemistry to **3** (2S, 3S, 6S), **4** (2S, 3S, 6S), **12** (2S, 3R, 6S) and **27** (2S, 3S, 6S), as shown



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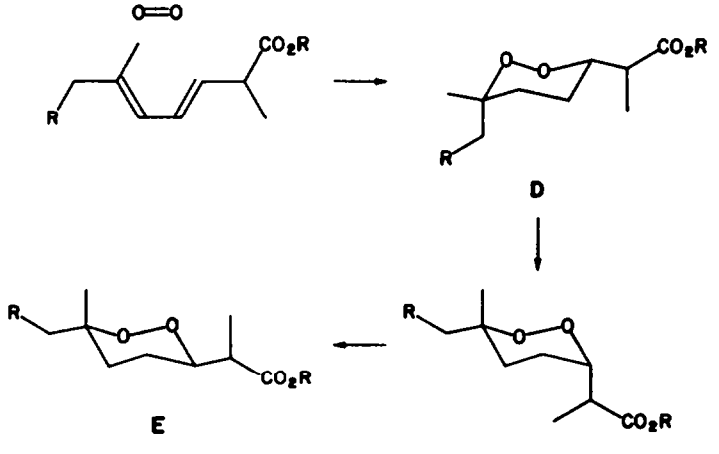
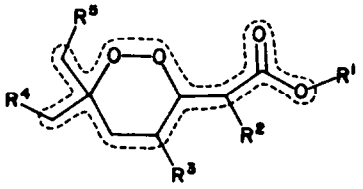
Attempts to obtain an authentic sample of muqubilin (11) in order to undertake a similar analysis of its absolute stereochemistry were unsuccessful However, a comparison of the optical rotation[16] for 11 (+31 6) with those of the methyl esters 9 (-56 5) and 12 (+52 6), which contain chiral peroxides of the same relative stereochemistry, suggests[23] that 11 has the same absolute stereochemistry as 12 (2S, 3R, 6S)

All the marine polyisoprenoid cyclic peroxides reported to date are norterpenes In trying to postulate a biosynthesis for the norterpene peroxides the assigned stereochemistries to C3 and C6 may be of significance In those examples where the C6 tertiary methyl is equatorial the stereochemistries assigned to C3 and C6 are the same (RR or SS) while the opposite holds where the C6 methyl is axial The former situation is consistent with the classical addition of oxygen across a trans, trans conjugated diene (D in Scheme 3) The latter can be rationalized by ring inversion to the alternate chair conformation and epimerization at C3 to the more stable equatorial orientation (E in Scheme 3) Enzymatic control would be required to impart absolute stereochemistry



SCHEME 3

Excluding the steroidal cyclic peroxides there exists a remarkable structural similarity between the known sponge cyclic peroxides[1-6] irrespective of their biosynthetic origins Polyisoprenoids and compounds of mixed (polyacetate, polypropionate etc) biogenesis all possess a common structural unit exemplified by 35 In each case the peroxy functionality is incorporated in a saturated six membered ring and is flanked by a quaternary and a tertiary carbon the latter being β to a carboxyl moiety There are a couple of exceptions[1a,g] to this generalization but even they show some of the features described above While most examples of this structural class are quite stable those possessing an allylic quaternary centre appear to rearrange readily to biologically inactive compounds on storage [1c] In general the free acids are reported to show significantly more antibiotic, antifungal or antimicrobial activity than the esters Structure vs activity studies on this class of compound are currently under investigation and will be reported elsewhere



35

EXPERIMENTAL

Collection, extraction and isolation

Sponge specimens were collected by hand (SCUBA) at a depth of 20 to 25 m The fresh specimens were immersed in ethanol, packed in dry ice, transported to the laboratory and stored at -20°C Type specimens were lodged with the Australian Museum and registry numbers allocated, Z4967 and Z4969

Evaporation of an ethanol extract of Z4967 (147 g dry wt) yielded a viscous pale yellow oil which was partitioned into CH_2Cl_2 (1 2 g), MeOH (8 4 g) and H_2O (3 4 g) soluble fractions The CH_2Cl_2 and MeOH fractions showed antimicrobial activity in a standard disc assay against Bacillus subtilis and Saccharomyces cerevisiae Elution of the CH_2Cl_2 solubles through Sephadex LH-20 with CH_2Cl_2 MeOH (1 1) yielded an interesting inactive component 12 (135 mgs, R_f 0 9 CH_2Cl_2 EtOAc [3 1] on silica) and an active two component mixture, 1 + 2 (450 mgs, R_f 0 1) Methylation of the latter fraction with diazomethane rendered it inactive but enabled it to be resolved by rapid elution (CH_2Cl_2 to EtOAc) through 5% $AgNO_3$ impregnated TLC grade silica into the methyl esters 3 (150 mgs) and 4 (186 mgs) (3 R_f 0 8, 4 R_f 0 1, CH_2Cl_2 EtOAc [3 1] on 2 5% $AgNO_3$ impregnated silica) The active components from the MeOH solubles were also identified as 1 and 2 and were isolated as their respective methyl esters 3 (80 mgs) and 4 (90 mgs) using the procedure described above

A similar extraction of Z4969 (173 g dry wt) with ethanol followed by solvent partitioning yielded antimicrobially active CH_2Cl_2 (234 mgs) and MeOH (7 g) soluble fractions Methylation with diazomethane followed by rapid elution through 5% $AgNO_3$ impregnated silica as described above yielded 27 (30 mgs) as the major metabolite (R_f 0 5, hexane EtOAc [4 1] on silica) Treatment of the MeOH solubles in a similar manner returned additional 27 (29 mgs)

Cyclic peroxide ester 3

A stable colourless oil (0 16% dry wt of Z4967) $[\alpha]_D^{25}$ -59 2° ($CHCl_3$, c 5 75), ^1H NMR ($CDCl_3$) δ 1 00 (6H, s), 1 13 (3H, s) 1 14 (3H, d, J=8 0 Hz), 1 61 (3H, s), 1 65 (3H, s), 2 57 (1H, dq, J=8 0, 8 0 Hz), 3 69 (3H, s), 4 24 (1H, bddd, J 8 0, 8 0, 4 0 Hz), 5 17 (1H, t, J=6 0 Hz), ^{13}C NMR ($CDCl_3$) 12 8 (q), 15 9 (q), 19 5 (t), 19 8 (q), 22 1 (t), 22 6 (t), 23 9 (q), 27 8 (t), 28 6 (2q), 32 4 (t), 32 7 (t), 34 8 (t), 34 9 (s), 39 8 (t), 40 2 (t), 42 6 (d), 51 8 (q), 79 9 (s), 81 3 (d), 123 5 (d), 126 9 (s), 136 2 (s), 137 1 ppm (s), 174.4 (s), EIMS m/z(%), 406 (M^+, 2), 388 (1), 375 (1), 357 (1), 319 (1), 301 (1), 137 (100),
HRMS 388 2956 (M^+-H_2O requires 388 2977, $C_{25}H_{40}O_3$), 319 2626 (M^+-$CH_3CHCO_2CH_3$ requires 319 2637, $C_{21}H_{35}O_2$)

Cyclic peroxide ester 4

A stable colourless oil (0 19 % dry wt of Z4967) $[\alpha]_D^{25}$-60.0° ($CHCl_3$, c 9 50), ^1H NMR ($CDCl_3$) δ 1 12 (3H, s) 1 14 (3H, d, J=8 0 Hz), 1 66 (9H, s), 1 74 (3H, s), 2.56 (1H, dq, J=8 0, 8 0 Hz), 3 69 (3H, s), 4 23 (1H, bddd, J=8 0, 8 0, 4 0 Hz), 5 23 (3H, m), ^{13}C NMR ($CDCl_3$) 12 7 (q), 15 9 (2q), 17 6 (q), 22 0 (t), 22 5 (t), 23 8 (q), 25 6 (q), 26 5 (t), 26 7 (t), 32.4 (t), 34 2 (t), 39 6 (2t), 42 5 (d), 51 7 (q), 79 8 (s), 81 2 (d), 124 1 (2d), 124 3 (d), 131 1 (s), 134 8 (s), 135 2 (s), 174.3 ppm (s), EIMS m/z (%), 406 (M^+, 2), 388 (1), 375 (1), 357 (1), 319 (1), 69 (100), HRMS 388 2956 (M^+-H_2O requires 388 2977, $C_{25}H_{40}O_3$), 319 2626 ($M^+-CH_3CHCO_2CH_3$ requires 319 2637, $C_{21}H_{35}O_2$), 319 2262 ($M^+-C_5H_9-H_2O$ requires 319 2273, $C_{20}H_{31}O_3$)

Cyclic peroxide ester 12

A stable colourless oil (0 09% dry wt of Z4967) $[\alpha]_D^{25}$ +52 2° ($CHCl_3$, c 5 65), ^1H NMR ($CDCl_3$) δ 1 26 (3H, s), 1 30 (3H, s) 1 60 (6H, s), 1 67 (3H, s), 2 65 (1H, dq, J=7 0, 7 0 Hz), 3 70 (3H, s), 4 12 (1H, bddd, J=8 0, 8 0, 4 0 Hz), 5 09 (2H, bt, J=8 0 Hz), ^{13}C NMR ($CDCl_3$) 13 5 (q), 15 9 (q), 17 6 (q), 20 5 (q), 21 6 (t), 23 4 (t), 25 6 (q), 26 5 (t), 31 9 (t), 39 6 (2t), 42 9 (d), 51 7 (q), 80 0 (s), 81 3 (d), 123 8 (d), 124 2 (d), 131 2 (s), 135 3 (s), 174 1 (s), EIMS m/z (%), 338 (M^+, 1), 323 (3), 320 (4), 251 (6), 69 (100), HRMS 338 2445 (M^+ requires 338 2457, $C_{20}H_{34}O_4$), 251 2019 ($M^+-CH_3CHCO_2CH_3$ requires 251 2011, $C_{16}H_{27}O_2$), 251 1661 ($M^+-C_5H_9-H_2O$ requires 251 1647, $C_{15}H_{23}O_3$)

Enantio sigmosceptrellin-A methyl ester 27

A stable colourless oil (0 08% dry wt of Z4969) $[\alpha]_D^{25}$ -57.1° ($CHCl_3$, c 0 7), ^1H NMR ($CDCl_3$) as reported[2] for sigmosceptrellin-A methyl ester (3), ^{13}C NMR ($CDCl_3$) 12 4 (q), 15 9 (q), 18 3 (q), 20 8 (q), 21 8 (t), 22 5 (t), 24 0 (q), 27 2 (t), 27 5 (t), 28 6 (t), 31 2 (t), 32 4 (t), 33 1 (t), 36 6 (d), 37 3 (t), 39 0 (s), 40 0 (s), 42 5 (d), 48 6 (d), 51 8 (q), 80 1 (s), 81 0 (d), 102 5 (t), 160 5 (s), HRMS 388 2956 (M^+-H_2O requires 388 2977, $C_{25}H_{40}O_3$)

LAD reduction of 3

To a solution of the cyclic peroxide 3 (35 mgs, 0 086 mmol) in dry ether (3 ml) was added excess $LiAlD_4$ (10 mgs, 0 24 mmol) and the resulting mixture stirred under reflux for 2 hrs The reaction was quenched by the addition of 10% aqueous HCl (2 ml) and extracted with EtOAc The EtOAc extract was then washed with H_2O, dried with anhydrous $MgSO_4$ and evaporated to yield the deuterated triol 14 (30 mgs, 91%) as a stable colourless oil ^1H NMR ($CDCl_3$) δ 0 86 (3H, d, J=7 0 Hz), 0 99 (6H, s), 1 21 (3H, s), 1 60 (3H, s), 1 68 (3H, s), 3 58 (1H, bm), 5 16 (1H, bt, J=6 5 Hz), EIMS m/z (%), 382 (M^+, 1), 364 (3), 349 (2), 145 (26), 137 (100), HRMS 364 3302 (M^+-H_2O requires 364 3310, $C_{24}H_{40}D_2O_2$)

LAD reduction of 4

Reduction of 4 (50 mgs) as described previously for 3 yielded the deuterated triol 15 (33 mgs, 70%) as a stable colourless oil ^1H NMR ($CDCl_3$) δ 0 86 (3H, d, J=8 0 Hz), 1 20 (3H, s), 1 60 (6H, s), 1 62 (3H, s), 1 68 (3H, s), 3 57 (1H, bm), 5 11 (3H, bm), EIMS m/z (%), 364 (M^+-H_2O, 27), 349 (5), 145 (100), HRMS 364 3298 (M^+-H_2O requires 364 3310, $C_{24}H_{40}D_2O_2$)

LAD reduction of 12

Reduction of 12 (30 mgs) as described previously for 3 yielded the deuterated triol 16 (22 mgs, 79%) as a stable colourless oil ^1H NMR ($CDCl_3$) δ 0 92 (3H, d, J=7 0 Hz), 1 20 (3H, s), 1 60 (3H, s), 1 62 (3H, s), 1 68 (3H, s), 3 80 (1H, bm), 5 08 (1H, bt, J=6 0 Hz), 5 14 (1H, bt, J=6 0 hz), EIMS m/z (%), 296 (M^+-H_2O, 9), 281 (2), 145 (27), 69 (100), HRMS 296 2682 (M^+-H_2O requires 296 2684, $C_{19}H_{32}D_2O_2$)

Isopropylidene derivative 17

The deuterated triol 14 (12 mgs) in DMF (1 ml) was treated with 2,2-dimethoxypropane (0 2 ml) and p-toluene sulfonic acid (2 mgs) and the resulting mixture stirred at room temperature overnight The H_2O quenched reaction was then extracted with EtOAc to yield the isopropylidene 17 (9 mgs, 68%) as a stable colourless oil ^1H NMR ($CDCl_3$) δ 0 76 (3H, d, J=8 0 Hz), 0 99 (6H, s), 1 17 (3H, s), 1 38 (3H, s), 1 43 (3H, s), 1 60 (3H, s), 1 66 (3H, s), 3.46 (1H, ddd, J=10 0, 8.0, 2 4 Hz), 5 17 (1H, bt, J=6 0 Hz), EIMS m/z (%) 422 (M^+, 1), 404 (6), 137 (100), HRMS 404 3623 (M^+-H_2O requires 404 3623, $C_{27}H_{44}D_2O_2$)

Isopropylidene derivative 18

Treatment of 15 (11 mgs) as described for 14 returned the deuterated isopropylidene 18 (10 mgs, 83%) as a stable colourless oil ^1H NMR ($CDCl_3$) δ 0 76 (3H, d, J=8 0 Hz), 1 16 (3H, s), 1 38 (3H, s), 1 43 (3H, s), 1 60 (6H, s), 1 62 (3H, s), 1 68 (3H, s), 3.57 (1H, ddd, J=10 0, 8 0, 2 4 Hz), 5 12 (3H, bm), EIMS m/z (%), 422 (M^+, 1), 404 (37), 145 (87), 137 (54), 121 (100), HRMS 404 3623 (M^+-H_2O requires 404 3632, $C_{27}H_{44}D_2O_2$)

Isopropylidene derivative 19

Treatment of 16 (10 mgs) as described for 14 returned the deuterated isopropylidene 19 (4 mgs, 35%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 1 07 (3H, d, J=7 0 Hz), 1 18 (3H, s), 1 39 (3H, s), 1 44 (3H, s), 1 60 (3H, s), 1.62 (3H, s), 1 68 (3H, s), 3 89 (1H, ddd, J=8 0, 4 0, 2.0 Hz), 5.11 (1H, bt, J=6 0 Hz), 5 14 (1H, bt, J=6.0 Hz), EIMS m/z (%), 336 (M^+-H_2O, 7), 137 (27), 69 (100), HRMS 336 3003 (M^+-H_2O requires 336 2997, $C_{22}H_{36}D_2O_2$)

LAD reduction of 8

Reduction of 8 (6 5 mgs) as previously described for 3 yielded the deuterated triol 20 (6 0 mgs, 98%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0.75 (3H, s), 0 80 (3H, d, J=8 0 Hz), 0 86 (3H, d, J=8.0 Hz), 1 04 (3H, s), 1 18 (3H, s), 3.47 (1H, bm), 4 51 (2H, s), EIMS m/z (%), 364 (M^+-H_2O, 1), 359 (1), 303 (10), 191 (60), 145 (100), HRMS 364.3306 (M^+-H_2O requires 364 3310, $C_{24}H_{40}D_2O_2$).

Isopropylidene derivative 21

Treatment of 20 (6.0 mgs) as described for 14 returned the deuterated isopropylidene 21 (5 mgs, 75%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 74 (3H, s), 0 76 (3H, d, J=8.0 Hz), 0 80 (3H, d, J=8 0 Hz), 1 04 (3H, s), 1 12 (3H, s), 1 38 (3H, s), 1 43 (3H, s), 3 46 (1H, ddd, J=10 0, 8 0, 2 5 Hz), 4 51 (2H, s), EIMS m/z (%), 407 (M^+-CH_3, 13), 389 (6), 203 (70), 191 (60), 145 (100), HRMS 407 3492 (M^+-CH_3 requires 407 3494, $C_{26}H_{43}D_2O_3$)

Hydrogenation of the cyclic peroxide 3

A sample of the cyclic peroxide 3 (13 mgs) in Et_2O with 10% Pd/C catalyst (10 mgs) were stirred under 1 atm of H_2 for 4 hrs. The catalyst was removed by filtration through celite and the product purified by stepwise elution (hexane to Et_2O) through a silica sep-pak to give the saturated diol ester 22 (8 mgs, 61%), as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 80 (3H, d, J=8 0 Hz), 0 86 (6H, s), 0 90 (3H, d, J=8 0 Hz), 1 17 (3H, s), 1 21 (3H, d, J=8 0 Hz), 2 56 (1H, dq, J=8 0, 8 0 Hz), 3 72 (3H, s), 3 70 (1H, bm), EIMS m/z (%), 394 (M^+-H_2O, 2), 379 (9), 376 (4), 307 (6), 171 (100), HRMS 394.3440 (M^+-H_2O requires 394 3447, $C_{25}H_{46}O_3$)

Hydrogenation of the cyclic peroxide 4

Hydrogenation of 4 (14 mgs) as described for 3 yielded the saturated ester 23 (8 mgs, 56%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 86 (12H, d, J=7 0 Hz), 1 17 (3H, s), 1 21 (3H, d, J=8 0 Hz), 2.56 (1H, dq, J=8 0, 8 0 Hz), 3 69 (1H, bm), 3.72 (3H, s), EIMS m/z (%), 396 (M^+-H_2O, 1), 381 (4), 378 (4), 309 (4), 171 (100), HRMS 381 3369 (M^+-H_2O-CH_3 requires 381 3332, $C_{25}H_{48}O_3$).

Hydrogenation of the cyclic peroxide 12

Hydrogenation of 12 (17 mgs) as described for 3 yielded the saturated diol ester 24 (8 mgs, 54%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 86 (9H, d, J=7 0 Hz), 1.17 (3H, s), 1.21 (3H, d, J=8.0 Hz), 2.56 (1H, dq, J=6 8, 8 0 Hz), 3.71 (3H, s), 3 88 (1H, ddd, J=8 0, 4.0, 4.0), EIMS m/z (%), 326 (M^+-H_2O, 2), 311 (5), 308 (4), 171 (100), HRMS 326 2812 (M^+-H_2O requires 326 2821, $C_{20}H_{38}O_3$)

Hydrogenation of the cyclic peroxide 9

Hydrogenation of 9 (10 mgs) as described for 3 yielded the saturated diol ester 25 (9 mgs, 89%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 72 (6H, d, J=8 0 Hz), 0.75 (6H, s), 1.16 (3H, s), 1.20 (3H, d, J=8 0 Hz), 2 56 (1H, bm), 3 71 (3H, s), 3 88 (1H, bm), EIMS m/z (%), 392 (M^+-H_2O, <1), 377 (1), 374 (2), 305 (2), 193 (100), 171 (39), HRMS 374.3195 (M^+-$2H_2O$ requires 374.3185, $C_{25}H_{42}O_2$)

Hydrogenation of the cyclic peroxide 27

Hydrogenation of 27 (7 mgs) as described for 3 yielded the saturated diol ester 28 (6 5 mgs, 92%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 70 (3H, d, J=8.0 Hz), 0 70 (3H, s), 0.76 (3H, s), 0.76 (3H, d, J=8.0 Hz), 1 17 (3H, s), 1 21 (3H, d, J=8 0 Hz), 2.55 (1H, dq, J=8.0, 8.0 Hz), 3.69 (1H, bm), 3 72 (3H, s), EIMS m/z (%), 377 (M^+-H_2O-CH_3, 2), 374 (2), 305 (5), 193 (72), 171 (100), HRMS 374 3195 (M^+-$2H_2O$ require 374.3184, $C_{25}H_{42}O_2$).

Acetylation of the diol ester 23

A sample of the diol ester 23 (60 mgs) in dry pyridine (1 ml) and acetic anhydride (0.5 ml) was stirred at room temperature overnight. The product obtained on addition of H_2O (0.5 ml) and evaporation to dryness was further purified by elution through a silica sep-pak with Et_2O to give the saturated acetoxy ester 29 (60 mgs, 91%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0.87 (12H, d, J=8 0 Hz), 1.15 (3H, s), 1 17 (3H, d, J=8.0 Hz), 2.04 (3H, s), 2 78 (1H, dq, J=8.0, 8.0 Hz), 3.68 (3H, s), 5 13 (1H, bm), EIMS m/z (%), 396 (M^+-AcOH, 1), 382 (10), 379 (9), 231 (11), 171 (100), 128 (50), HRMS 395 3529 (M^+-$C_2H_5O_2$ requires 395 3526, $C_{25}H_{47}O_3$)

Dehydration of the acetoxy ester **29**

A solution of the acetoxy ester **29** (60 mgs) in dry benzene (5 ml) was treated with p-toluene sulphonic acid (10 mgs) and the mixture stirred under reflux conditions for 2 hrs Evaporation of the benzene followed by extraction with hexane returned the unsaturated acetoxy ester **30** (48 mgs, 83%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 87 (12H, d, J=8 0 Hz), 1 17 (3H, d, 8 0 Hz), 2 04 (3H, s), 2 78 (1H, dq, J=8 0, 8 0 Hz), 3 68 (3H, s), 5 13 (2H, bm), EIMS m/z (%), 407 (M^+-OCH_3, 2), 378 (60), 254 (20), 195 (30), 171 (100), HRMS 407 3523 (M^+-OCH_3 requires 407 3525, $C_{26}H_{47}O_3$)

Hydrogenation of **30**

The unsaturated acetoxy ester **30** (48 mgs) was hydrogenated as described for **3** to yield the saturated acetoxy ester **31** (42 mgs, 87%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 87 (15H, m), 1 15 (3H, d, J=8 0 Hz), 2 08 (3H, s), 2 77 (1H, dq, J=7 0, 7 0 Hz), 3 68 (3H, s), 5 10 (1H, bm), EIMS m/z (%), 440 (M^+, 1), 409 (5), 397 (10), 380 (70), 297 (20), 266 (15), 171 (100), HRMS m/z (%), 440 3866 (M^+ requires 440 3865, $C_{27}H_{52}O_4$)

Hydrolysis of **31**

The acetoxy ester **31** (40 mgs) in MeOH (1 ml) with K_2CO_3 was stirred overnight at room temperature Evaporation of the MeOH followed by addition of 10% aqueous HCl and extraction with EtOAc yielded the crude acid **32** which was methylated with diazomethane HPLC [8 mm radial pak 10μ silica column, elution with 2 5% EtOAc in hexane] yielded the pure hydroxy ester **33** (20 mgs, 53%) as a stable colourless oil ^{1}H NMR ($CDCl_3$) δ 0 84 (6H, d, J=8 0 Hz), 0 86 (9H, d, J=8 0 Hz), 1 20 (1 5H, d, J=7 0 Hz), 1 21 (1 5H, d, J=7 0 Hz), 2 54 (1H, dq, J=8 0, 8 0 Hz), 3 69 (1H, bm), 3 71 (3H, s), EIMS m/z (%), 398 (M^+, 1), 383 (2), 380 (15), 310 (2), 171 (25), 88 (100), HRMS 398 3740 (M^+ requires 398 3760, $C_{25}H_{50}O_3$) When the hydrolysis of **31** was carried out in MeOD (1 ml) with K_2CO_3 a partially deuterated hydroxy ester was obtained EIMS m/z (%) 89 (40), 88 (100)

Horeau determinations - general procedure

To an accurately measured amount of the secondary alcohol to be analysed was added a two fold excess of α-phenylbutyric anhydride (as a 12 5% solution in dry pyridine) and the mixture stirred for 48 hrs at room temperature Excess anhydride was then quenched by the addition of H_2O (~5 ml) and the reaction mixture titrated against 0 005N NaOH (phenolphthalein indicator) to determine the amount of free α-phenylbutyric acid and hence percentage esterification After extraction of the ester the reaction mixture was acidified and the partially resolved α-phenylbutyric acid extracted with benzene $[\alpha]_D^{25}$ measurements on the material established the nature of the enantiomeric excess and thus the absolute stereochemistry of the secondary alcohol Results for Horeau calculations on derivatives of **3**, **4**, **9**, **12** and **27** are displayed in Table 2

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NOTE ADDED IN PROOF Specimens Z4967 and Z4969 have been identified as a Latrunculia sp (Latrunculiidae, Poecilosclerida [incertae sedis] and Mycale (aegogrophila) c f ancorina (Whitelegge, 1906, p 466) (Mycalidae, Poecilosclerida) respectively, by J Hooper at the Northern Territory Museum of Arts and Sciences, Darwin, Australia