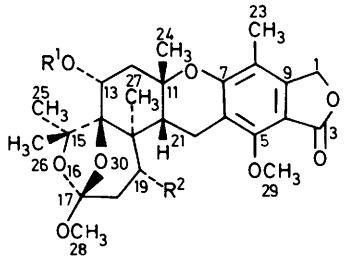
Metabolites of *Aspergillus ustus*. Part 1. Application of the Heteronuclear Selective Population Inversion (SPI) N.M.R. Technique to the Structure Elucidation of the Austalides A—F, Novel Ortho Ester Meroterpenoids[1]

R. Marthinus Horak,* Pieter S. Steyn, and Robert Vleggaar
National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa
Christiaan J. Rabie
National Research Institute for Nutritional Diseases, Medical Research Council, P.O. Box 70, Tygerberg 7505, Republic of South Africa

The isolation and characteristics of 12 biosynthetically related metabolites, austalides A—L from cultures of *Aspergillus ustus* are reported. The structure elucidation of the austalides A—F is based on a detailed study of their high-field ^1H and ^{13}C n.m.r. spectra and especially on the three- and two-bond (C,H) connectivity pattern as determined by heteronuclear ^{13}C-{^1H} selective population inversion (SPI) experiments. The conformation and relative configuration of austalides A and D were deduced from the observed proton–proton nuclear Overhauser effects (n.O.e.s) and the magnitude of the proton–proton coupling constants. Base-catalysed hydrogen–deuterium exchange on the diketone derivative (**18**), obtained by Jones oxidation of austalide F, leads to the regio- and stereo-specific incorporation of deuterium atoms at C-12, C-18, and C-21. A mechanism is proposed to explain the incorporation of a deuterium atom at C-21, a position γ to the C-13 carbonyl group.

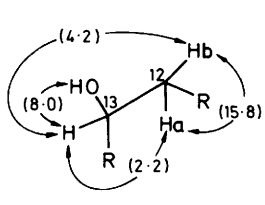
Previous investigations of toxigenic cultures of *Aspergillus ustus* resulted in the isolation of a number of mycotoxins, *viz.* the austamides,[2] austdiol,[3] the austocystins,[4] and austin.[5] In the present study a highly toxigenic strain of *Aspergillus ustus* (Bainier) Thom. and Church (strain MRC 1163), isolated from dried fish destined for human consumption in the Bandar-Shah region of Iran was investigated. Extraction of toxic maize meal cultures with chloroform–methanol followed by fractionation of the toxic extract, led to the isolation of the known pigments averufin[6] and versicolorin C,[7] the austocystins A, B, D, and H,[4] as well as a number of new derivatives of versicolorin A and austocystin A.[8] In addition, a range of novel biosynthetically related meroterpenoid metabolites, the austalides A—L, were isolated. The isolation of the austalides A—L and the structure elucidation of austalides A—F [(**1**)—(**6**)] based on data obtained by high-field ^1H and ^{13}C n.m.r. spectroscopy as well as chemical derivation form the subject of this paper.



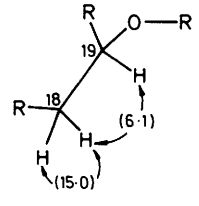
(**1**) A : R^1 = $^{33}COCH_3$ ^{34} R^2 = H
(**2**) B : R^1 = H R^2 = H
(**3**) C : R^1 = $COCH_3$ R^2 = $O^{31}C^{32}OCH_3$
(**4**) D : R^1 = H R^2 = $OCOCH_3$
(**5**) E : R^1 = $COCH_3$ R^2 = OH
(**6**) F : R^1 = H R^2 = OH

The structure of austalide D (**4**), the major metabolite in the austalide series, was investigated first. The compound crystallized from acetone as colourless prisms, m.p. 259—261 °C and elemental analysis indicated the molecular formula $C_{28}H_{36}O_{10}$. Absorptions at $\lambda_{max.}$ 222 and 268 nm (ε 32 700 and 17 700, respectively) are consonant with the presence of the substituted phthalide moiety in the molecule. The i.r. spectrum exhibited an absorption maximum at 1 745 cm^{-1}, due to the phthalide and acetate carbonyl groups (C-3 and C-31, respectively).[9] The ^1H and ^{13}C n.m.r. data of austalide D are collated in the Table. Six three-proton singlet signals (δ_H 0.964, 1.258, 1.488, 1.771, 2.019, and 2.041) were assigned to the protons of the tertiary C-methyl groups, whereas two three-proton singlets at δ_H 3.378 and 4.119, could be assigned to the protons of two methoxy groups. The two-proton singlet signal at δ_H 5.087 was indicative of the oxygen-substituted benzylic methylene group, C-1.[10]

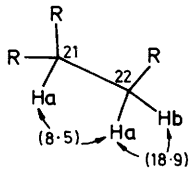
The remainder of the signals of the ^1H n.m.r. spectrum of austalide D exhibited fine structure. First-order analyses of these multiplets yielded the values of the proton chemical shifts and proton-proton coupling constants. From the values of the coupling constants, as corroborated by ^1H-{^1H} homonuclear decoupling experiments, three fragments A, B, and C of the austalide D molecule could be constituted as shown in (**7**)—(**9**).



(**7**)



(**8**)



(**9**)

Fragment A (**7**). The chemical-shift value of the methine proton, 13-H (δ_H 4.184), indicated that it is situated on an oxygen-bearing carbon atom. The presence of the hydroxy

Table. ^{13}C and ^{1}H N.m.r. data of austalides D (**4**) and A (**1**)

	(4)		(1)	(4)		(1)	
Carbon	δ_C/p.p.m.	^1J (CH)/Hz	δ_C/p.p.m.	δ_H/p.p.m.	J (HH)/Hz	δ_H/p.p.m.	J (HH)/Hz
1	68.10 T	151.3	68.04 T	5.087 s		5.070 s	
3	168.93 S		169.13 S				
4	108.63 S		107.27 S				
5	155.61 S		155.23 S				
6	116.26 S		115.57 S				
7	156.58 S		157.79 S				
8	114.10 S		113.79 S				
9	145.76 S		145.53 S				
11	78.23 S		75.48 S				
12	40.84 T	128.7	38.00 T	2.399 dd	15.8, 2.2	2.530 dd	16.1, 2.2
				2.320 dd	15.8, 4.2	2.136 dd	16.1, 4.4
13	69.92 D	148.2	70.84 D	4.184 m	4.2, 2.2, 8.0[a]	5.067 dd	4.4, 2.2
14	86.10 S		85.33 S				
15	85.69 S		84.41 S				
17	117.47 S		119.24 S				
18	37.34 T	131.9	30.28 T	2.274 dd	15.0, 6.1	1.822—	
				1.935 d	15.0	1.946 m	
19	71.07 D	150.4	30.56 T		6.1		
20	44.98 S		40.35 S				
21	38.49 D	128.7	35.86 D	2.176 d	8.5	2.398 d	8.2
22	19.73 T	130.1	17.92 T	3.229 d	18.9	2.930 d	18.7
				2.975 dd	18.9, 8.5	2.827 dd	18.7, 8.2
23	10.61 Q	128.1	10.36 Q	2.019 s		2.030 s	
24	27.35 Q	126.8	27.39 Q	1.258 s		1.213 s	
25	25.73 Q	127.6	25.81 Q	1.771 s		1.569 s	
26	29.65 Q	129.1	28.45 Q	1.488 s		1.318 s	
27	14.07 Q	129.7	17.95 Q	0.964 s		0.970 s	
28	48.17 Q	144.1	48.69 Q	3.378 s		3.417 s	
29	62.16 Q	146.1	61.78 Q	4.119 s		4.087 s	
31	170.27 S						
32	21.13 Q	129.8		2.041 s			
33			169.31 S				
34			21.01 Q			1.960 s	

[a] The proton of 13-OH gives rise to a doublet (J 8.0 Hz) at δ_H 2.603.

function at C-13 was inferred from the disappearance of the signal at δ_H 2.603 (J 8.0 Hz) and the removal of a 8.0 Hz coupling from the 13-H resonance on addition of deuterium oxide to the sample.

Fragment B (**8**). The signal of the methine proton, 19-H appears at low-field (δ_H 5.433) due to the oxygen substituent at C-19. This oxygen function must be either an ester or an ether, as addition of deuterium oxide to the sample did not change the 19-H resonance in the ^1H n.m.r. spectrum.

Fragment C (**9**). The chemical shifts and geminal coupling constant (J 18.9 Hz) of 22-H are indicative of benzylic methylene protons. The lack of coupling between one of these protons and the contiguous 21-H indicates a torsion angle of close to 90° between these vicinal protons.[11]

The ^{13}C n.m.r. data of austalide D (Table), obtained from single frequency n.O.e. ^{13}C n.m.r. spectra, revealed that the 28 carbon signals observed in the broad-band proton-decoupled ^{13}C spectrum are due to 8 methyl, 4 methylene, 3 methine, and 13 quaternary carbon atoms. The residual splittings observed in a series of off-resonance proton-decoupled ^{13}C n.m.r. experiments allowed the correlation of the signals of the proton-bearing carbon atoms with specific proton resonances.[12] These results also allowed the assignment of the proton-bearing carbon atoms in fragments A (**7**), B (**8**), and C (**9**). The magnitude of the observed, directly bonded (C,H) coupling constants (Table) support these assignments.[13]

The connections between the different structural units in austalide D were determined by heteronuclear ^{13}C-$\{^1H\}$ selective population inversion (SPI) experiments utilizing the two- and three-bond (C,H) couplings.[14] Interpretation of the SPI experiments was facilitated by using difference SPI spectroscopy in which a control spectrum is subtracted from the perturbed spectrum so that only differences between the two spectra are observed[15] (see Figure 1).

The protons of the tertiary methyl group, C-27, served as the starting point for the assembly of the structure of austalide D. The three-proton signal at δ_H 0.964, correlated with the resonance at δ_C 14.07 in the ^{13}C n.m.r. spectrum, is assigned to the C-27 protons on the basis of the chemical-shift value. Application of a π-pulse with $\gamma H_2 = 5$ Hz at a position 5 Hz to low-field of the 27-H resonance in a ^{13}C-$\{^1H\}$ SPI experiment affected the ^{13}C resonances at δ_C 38.49 D, 44.98 S, 71.07 D, and 86.10S (see Figure 1). The tertiary nature of the C-27 methyl group requires that it should be located on the carbon atom (C-20) which resonates at either δ_C 44.98 or δ_C 86.10, the latter being indicative of an oxygen-substituted carbon atom. This quaternary carbon atom (C-20) in turn must be linked to three other carbon atoms in order to explain the two- and three-bond (C,H) coupling connectivity pattern determined in the SPI experiment. The alternative arrangement in which an oxygen substituent is located at C-20 necessitates a four-bond (C,H) coupling between the methyl protons and the carbon atom which resonates at δ_C 44.98. Four-bond (C,H) couplings are normally small (*ca.* 1 Hz)[16] and the irradiating power used in the SPI experiments precludes their detection. As a consequence the resonance at δ_C 44.98 must be due to C-20, a quaternary

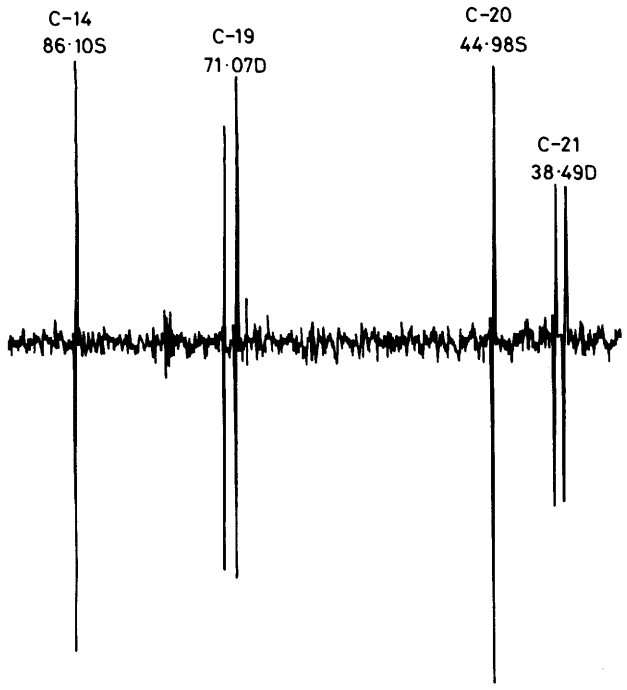
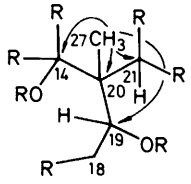


Figure 1. Difference ^{13}C-{^{1}H} SPI spectrum of austalide D (**4**) obtained on application of a π-pulse (γH_2 = 5 Hz) to the 27-H proton transitions

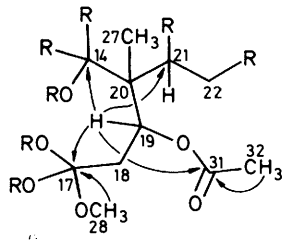
carbon atom two bonds removed from the C-27 protons whereas the resonances at δ_C 38.49 (C-21), 71.07 (C-19), and 86.10 (C-14) must be three bonds removed. The signals of the two proton-bearing carbon atoms C-19 (δ_C 71.07 D) and C-21 (δ_C 38.49 D) have been correlated with specific proton resonances in fragments B (**8**) and C (**9**), respectively, and the partial structure (**10**) can therefore be formulated.



(**10**)

A SPI experiment on the C-19 proton transitions (δ_H 5.433) affected the resonances assigned to C-14 (δ_C 86.10) and C-21 (δ_C 38.49), as well as those at δ_C 170.27 S (C-31) and δ_C 117.47 S (C-17). The carbon atoms C-31 and C-17 must both be three bonds removed from 19-H and each can therefore be linked to either C-18 or the C-19 oxygen function. This ambiguity was resolved in the following manner. Chemical-shift considerations dictate that the resonance at δ_C 170.27 (C-31) must be due to a sp^2 carbon atom of a carbonyl group linked to an oxygen function. Selective irradiation of the 32-H signal (δ_H 2.041, 3 H, s) in a SPI experiment proved that these protons are either two or three bonds removed from C-31. The carbon chemical shift of the C-32 methyl group, δ_C 21.13 (correlated with the chemical shift of the 32-H resonance) is indicative of the methyl carbon atom of an *O*-acetyl group. The two- and three-bond (C,H) connectivity pattern demanded by the results of the above two SPI experiments, as well as chemical shift requirements, are satisfied only by the partial structure (11).

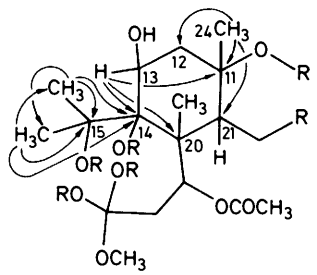
The location of a methoxy group at C-17 followed from the three-bond (C,H) coupling observed between the *O*-methyl protons (28-H, δ_H 3.378) and C-17 upon selective irradiation of



(**11**)

these protons in a SPI experiment. The chemical shift of C-17 (δ_C 117.47) indicates that it is substituted by an additional two oxygen atoms to form an ortho ester moiety as shown in (**11**). The reported chemical shifts of the carbon atom of ortho esters are in the 117—119 p.p.m. region in good agreement with the chemical shift found for C-17 in (**11**).[17]

Selective irradiation of the 24-H methyl proton transitions affected the resonances assigned to C-21 (δ_C 38.49 D) as well as those at δ_C 40.84 T and 78.23 S. The resonance at δ_C 40.84 has been correlated with the resonance of the 12-H protons in fragment A (**7**) and is, therefore, assigned to C-12. The (C,H) couplings to C-12 and C-21 must be over three bonds since the corresponding proton resonance of this tertiary methyl group is a singlet. As a result of this experiment a two-carbon unit consisting of a methyl group located on a quaternary carbon atom, C-11, must connect C-12 of fragment A (**7**) with C-21 in the partial structure (**11**). The quaternary carbon atom concerned resonates at δ_C 78.23 since this resonance is also affected by irradiation of the 13-H proton transitions in a ^{13}C-{^{1}H} SPI experiment. The chemical shift of the C-11 resonance is characteristic of an oxygen-bearing carbon atom. In addition to the above, the results obtained from the SPI experiments indicated a two- and three-bond (C,H) connectivity pattern between 13-H and the carbon atoms C-14 and C-20, respectively, thereby leading to the linkage between C-13 and C-14 [see partial structure (**12**)].



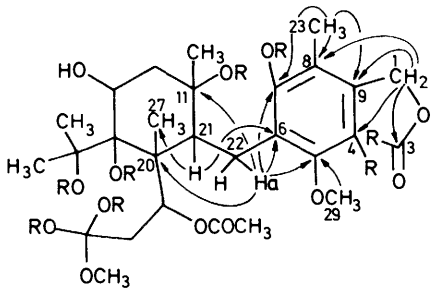
(**12**)

The presence of a geminal dimethyl moiety in austalide D as shown in (**12**) was demonstrated by the following results. Selective irradiation of the proton transitions of either methyl group (25-H : δ_H 1.771; 26-H : δ_H 1.488) affected the signal assigned to the carbon atom of the other methyl group (C-25 : δ_C 25.73; C-26 : δ_C 29.65) as well as the quaternary carbon resonance at δ_C 85.69 (C-15). The proximate location of this three carbon moiety to C-14 was evident from the three-bond (C,H) coupling between the protons of both the C-25 and C-26 methyl groups and C-14 (δ_C 86.10). The chemical shift of the quaternary carbon resonance at δ_C 85.69 (C-15) is indicative of the presence of an oxygen function at this carbon.

Chemical-shift considerations dictate that the remaining seven singlet resonances in the δ_C 108—169 region of the ^{13}C n.m.r. spectrum of austalide D must be attributed to the sp^2 carbon atoms of a completely substituted benzene ring and an

ester carbonyl carbon atom (δ_C 168.93). Two of the substituents on the aromatic ring are identified as oxygen atoms by the resonances at δ_C 155.61 (C-5) and 156.58 (C-7).

Evidence for the linkage of the partial structure (**12**) and the aromatic nucleus in austalide D (**4**) was provided by selective irradiation of the 22-H_a transitions in a SPI experiment. The results show that 22-H_a couples to the carbon atoms two- and three-bonds removed, three of which resonate at δ_C 116.26 (C-6), 155.61 (C-5), and 156.58 (C-7) [*cf.* partial structure (**13**)]. The 21-H proton must be three bonds removed from C-6 since the resonance at δ_C 116.26 (C-6) is also affected when the C-21 proton transitions are irradiated. In addition, the chemical-shift values (δ_H 3.229 and 2.975), the difference in chemical shift ($\Delta\delta$ 0.254 p.p.m.), as well as the value of the geminal (H,H) coupling constant (J 18.9 Hz) of 22-H_a and 22-H_b indicated that these protons are situated in close proximity to the aromatic ring in austalide D.



(**13**)

Selective irradiation of the transitions due to the *O*-methyl protons, 29-H, in a SPI experiment affected the signal of the oxygen-bearing carbon atom three bonds removed, C-5, (δ_C 155.61). Results from a SPI experiment indicated the presence of two- and three-bond (C,H) couplings between the methyl protons, 23-H, and the carbon atoms which resonate at δ_C 114.10, 145.76, and 156.58 (C-7). The coupling of 23-H with the oxygen-substituted, sp^2 carbon atom, C-7 is through necessity over three bonds and the C-23 methyl group must, therefore, be located at C-8, as illustrated in (**13**).

It is of importance to note that the resonances at δ_C 114.10 and 145.76 (due to C-8 and C-9) are affected when the transitions of either the C-23 or the C-1 protons are irradiated in a SPI experiment. The C-23 methyl group and the C-1 methylene group therefore have the *ortho* orientation. The above SPI experiment also indicated (C,H) coupling between 1-H and the carbon atoms which resonate at δ_C 108.63 and 168.93. The low-field chemical shift values of the ^1H and ^{13}C n.m.r. signals of the C-1 methylene group (δ_H 5.087 and δ_C 68.10, respectively) indicate that it carries an oxygen substituent. The magnitude of the one-bond (C,H) coupling constant of C-1 [J (CH) 151.3 Hz] is in agreement with this assignment.[13]

The formation of the carbon–carbon linkage between C-3 and C-4 in the partial structure (**13**) follows from chemical-shift considerations. Inspection of a Dreiding model of (**13**) clearly indicates that the remaining carbon-oxygen linkages can be formed in only one way which leads to the constitution of austalide D as depicted in structure (**4**).

The relative configuration of austalide D was deduced from the magnitude of the proton–proton coupling constants as well as the proton–proton nuclear Overhauser effects (n.O.e.s) (see Figure 2). For the basis of the discussion it is assumed that C-11 has the *S*-configuration, *i.e.* the C-24 methyl group is situated above the plane of ring D. An appreciable n.O.e. is observed between 24-H and the protons which resonate at δ_H 2.176 (21-H) and δ_H 2.975 (22-H_b). This result establishes that rings C and D are *cis*-fused.

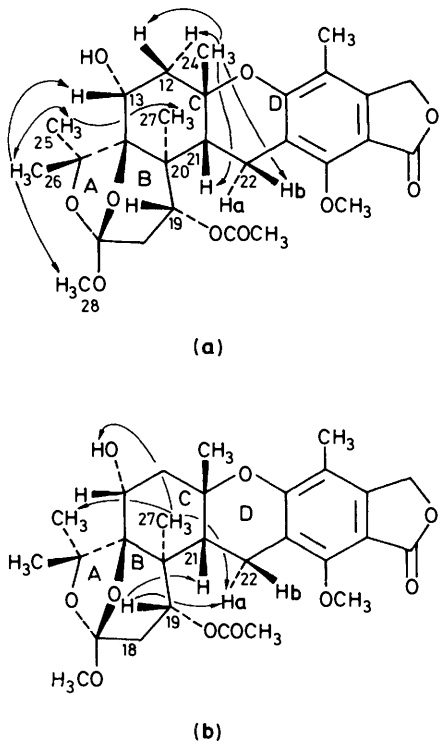


Figure 2. Proton–proton n.O.e. connectivity pattern of austalide D (**4**)

The fact that an n.O.e. is observed between the C-27 protons and 22-H_a, but not for 27-H and 21-H, indicates a *trans* relationship between the C-27 and C-24 methyl groups. The n.O.e. between the C-27 and C-25 protons shows that these two methyl groups are *cis* orientated. Consequently, rings B and C must be *cis*-fused and the seven-membered ring and ring C *trans*-fused. This result incidentally also allows the unambiguous assignment of the signals at δ_C 25.73 and δ_C 29.65 to C-25 and C-26, respectively, since the signals of the proton-bearing atoms in the molecule have been correlated with specific proton resonances. This assignment was of vital importance to the study of the biosynthesis of the austalides,[18] since it allowed the formulation of a plausible mechanism for the formation of austalide D, consistent with the relative stereochemistry of these metabolites.

The secondary hydroxy group at C-13 lies below the plane of ring C as an n.O.e. is observed between the protons of the C-27 methyl group and the hydroxy proton. The n.O.e. observed for 13-H upon irradiation of the C-26 protons confirms the relative configuration for C-13. The n.O.e.s between 19-H and 21-H, and 22-H_a can be explained if the C-19 acetoxy function lies below the plane of ring B.

On the basis of the above results the relative configuration as depicted in Figure 2, *i.e.* 11*S*, 13*R*, 14*R*, 17*S*, 19*R*, 20*S*, and 21*R*, or the enantiomer thereof, is assigned to austalide D. The enantiomeric configuration would result if the 11*R*-configuration is used.

The molecular formulae, as determined by elemental analyses and mass spectrometry, for austalides C (**3**) ($C_{30}H_{38}O_{11}$), D (**4**) ($C_{28}H_{36}O_{10}$), E (**5**) ($C_{28}H_{36}O_{10}$) and F (**6**) ($C_{26}H_{34}O_9$) indicate that these compounds share the same basic structure and differ from each other only in the nature of the substituents at certain carbon atoms. This supposition is borne out by the u.v. characteristics of these metabolites.

The structure of austalide C (**3**) should, therefore, differ from that of austalide D (**4**) only by the presence of an *O*-acetyl group which through necessity must be located at C-13. This

assumption was confirmed by a comparison of the ^{1}H n.m.r. data for the two metabolites. The presence of an additional *O*-acetyl group in austalide C was evident from the three-proton singlet at δ_H 1.966 and the downfield shift of 0.936 p.p.m. observed for the 13-H resonance upon acetylation. A comparison of the ^{13}C n.m.r. data showed that acetylation causes a downfield shift ($\Delta\delta$ 0.80 p.p.m.) for the C-13 resonance (δ_C 69.92) and an upfield shift ($\Delta\delta$ −3.01 p.p.m.) for the resonance of the adjacent methylene carbon atom, C-18 (δ_C 37.34) in austalide C. These findings prove that austalide C (**3**) is the 13-*O*-acetyl derivative of austalide D.

Alkaline hydrolysis (0.1M-potassium hydroxide in methanol) of austalides D and C resulted in each case in the formation of austalide F (**6**). The ^{1}H n.m.r. spectrum of austalide F showed two signals at δ_H 2.710 and 3.051, assigned to the protons of the C-13 and C-19 hydroxy groups, which disappeared upon addition of deuterium oxide to the sample. This experiment also led to the sharpening of the multiplets due to 13-H (δ_H 4.131) and 19-H (δ_H 3.922). The latter chemical shift corresponds to an upfield shift ($\Delta\delta$ −1.511 p.p.m.) of the 19-H resonance upon hydrolysis of the 19-*O*-acetyl group in austalide D. The differences observed in the ^{13}C n.m.r. data for austalides D and F are consistent with the proposed structures.

Austalides D (**4**) and E (**5**) share the same molecular formula and it is evident from a comparison of their ^{1}H and ^{13}C n.m.r. data that they differ only in the location of the *O*-acetyl group. The facile transformation of austalide E into C (**3**) upon treatment with acetic anhydride and pyridine or into austalide F (**6**), using 0.1 M-potassium hydroxide in methanol, proved the interrelationship between these metabolites.

Austalide A (**1**), $C_{28}H_{36}O_9$·$CHCl_3$, has the characteristic u.v. absorptions assigned to the phthalide chromophore in the austalides C–F [(**3**)—(**6**)]. The structure elucidation of austalide A followed the procedure outlined for austalide D (**4**). The ^{1}H and ^{13}C n.m.r. data of the metabolite are collated in the Table. The signals of the proton-bearing carbon atoms C-1, C-13, C-21, C-23, C-24, C-25, C-26, C-27, C-28, C-29, and C-34 were correlated with specific proton resonances using the residual (C,H) couplings observed in a series of off-resonance proton-decoupled ^{13}C n.m.r. experiments. The presence of certain structural units, recognized from an analysis of the ^{1}H n.m.r. spectrum, was confirmed by ^{1}H-{^{1}H} decoupling experiments. The connection between the different units was determined by heteronuclear ^{13}C-{^{1}H} SPI experiments utilizing two- and three-bond (C,H) couplings (see Figure 3).

A comparison of the molecular formulae of austalides A (**1**) ($C_{28}H_{36}O_9$) and E (**5**) ($C_{28}H_{36}O_{10}$) indicates that austalide A lacks an oxygen atom. The ^{1}H n.m.r. spectrum of austalide A lacks the resonance at δ_H 4.020, assigned to the methine proton of the hydroxy-bearing carbon atom, C-19, in austalide E (**5**). The ^{13}C n.m.r. spectrum of austalide A showed only one resonance [δ_C 70.84, *J*(CH) 146.7 Hz] characteristic of an oxygen-bearing methine carbon atom (C-13) whereas two such resonances are present in the spectrum of austalide E. Furthermore, a resonance at δ_C 30.56 [*J*(CH) 129.1 Hz], not present in the ^{13}C n.m.r. spectrum of austalide E, is assigned to a sp^3 methylene group. It was therefore postulated that austalide A is the 19-deoxy analogue of austalide E and consequently that the *O*-acetyl group is situated at C-13. This postulate was substantiated by the three-bond (C,H) coupling pattern between 13-H and C-33 on selective irradiation of the C-13 proton transitions in a ^{13}C-{^{1}H} SPI experiment (see Figure 3). Additional evidence in favour of the 19-deoxy structure (**1**) was provided by the (C,H) coupling pattern of the 27-H protons.

The relative configuration of austalide A was deduced from the observed proton–proton n.O.e.s (see Figure 4) using similar arguments as described for austalide D as well as single crystal *X*-ray crystallography.[1]

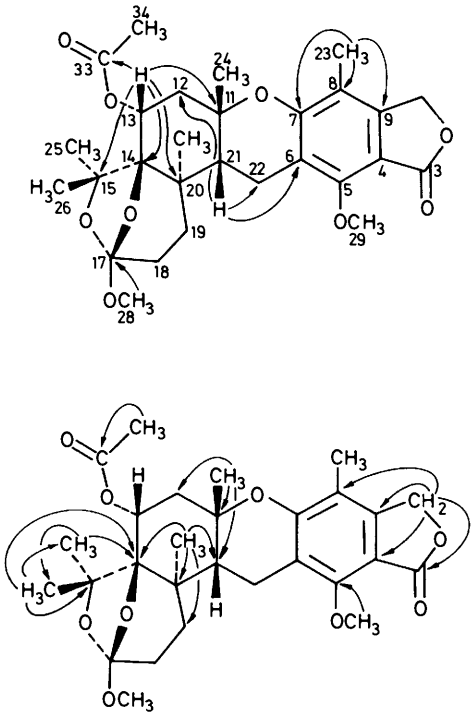


Figure 3. The (C,H) connectivity pattern of austalide A (**1**) determined by ^{13}C-{^{1}H} SPI experiments

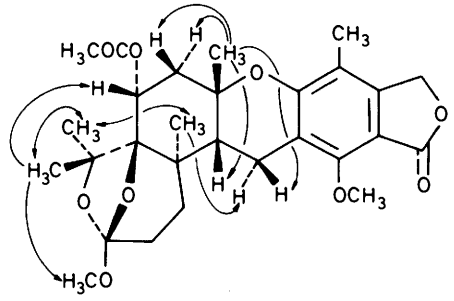


Figure 4. The n.O.e. connectivity pattern of austalide A (**1**)

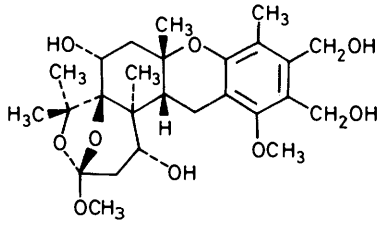
Austalide B (**2**), $C_{26}H_{34}O_8$, had a molecular mass of 474, 42 mass units less than austalide A (**1**). A comparison of the ^{1}H n.m.r. spectra of austalides A (**1**) and B (**2**) showed a characteristic upfield shift for the 13-H signal on change of the C-13 substituent from an acetoxy to a hydroxy group. A similar chemical shift difference ($\Delta\delta$ 0.931 p.p.m.) was observed for the 13-H protons of austalides D (**4**) and E (**5**). The differences in the chemical shifts of the carbon atoms C-12, C-13, and C-14 in austalides A and B can be rationalized in a similar manner.

Alkaline hydrolysis of austalide A led to its facile conversion into austalide B.

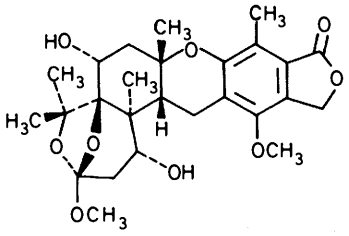
In the course of the structure elucidation of the austalides a number of chemical reactions were performed on various of the metabolites to obtain additional information on their constitution. The results provide an insight into the chemistry of the austalides.

Lithium aluminium hydride reduction of austalide D (**4**) yielded austalide F (**6**) and the bis(hydroxymethyl) compound (**14**). The latter has u.v. maxima at 228, 281, and 288 nm and lacked the characteristic i.r. absorption band at 1 745 cm^{-1}, assigned to the phthalide and *O*-acetyl carbonyl groups in the austalides. The ^{1}H n.m.r. spectrum exhibited a two-proton

singlet at δ_H 4.763 due to the C-1 methylene protons. A pair of doublets (J 11.8 Hz) at δ_H 4.782 and 4.824 were attributed to the C-3 protons. These assignments are based on the assumption that the *O*-methyl group (C-29) rather than the C-methyl group (C-23) in (**14**) causes the magnetic non-equivalence of the protons of the contiguous benzylic methylene group.



(**14**)

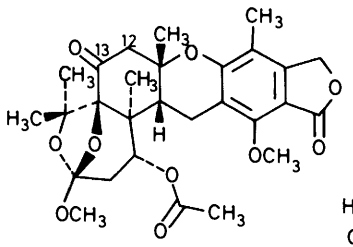


(**15**)

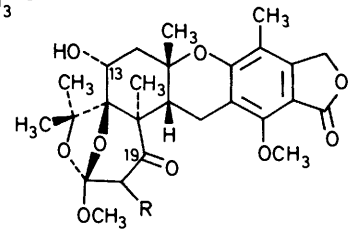
Oxidation of the bis(hydroxymethyl) compound (**14**) with barium manganate afforded a 1 : 1 mixture of austalide F (**6**) and its isomer (**15**). A comparison of the ^{1}H n.m.r. spectra of these two isomers supported the above structural conclusions. In the spectrum of (**15**) the signal due to the C-29 *O*-methyl protons (δ_H 3.902) showed an upfield shift ($\Delta\delta$ −0.224 p.p.m.) whereas the protons of the C-23 methyl group resonated at considerably lower field ($\Delta\delta$ 0.414 p.p.m.)[19] as a result of the anisotropy of the C-1 carbonyl group.

Oxidation of austalide D (**4**) with Jones reagent yielded the ketone (**16**). The ^{1}H n.m.r. spectrum of (**16**) exhibited an AX spin system (J 12.0 Hz) ascribed to the C-12 methylene protons (δ_H 2.683 and 3.422). Jones oxidation of austalide F (**6**) yielded the monoketone (**17**) and the diketone (**18**). The ^{1}H n.m.r. spectrum of the monoketone showed an AX spin system assigned to the C-18 methylene protons (δ_H 2.724 and 2.956, J 15.8 Hz) whereas two AX spin systems are apparent in the spectrum of (**18**) and are assigned to 12-H (δ_H 2.805 and 3.460, J 12.7 Hz) and 18-H (δ_H 2.808 and 3.143, J 15.8 Hz). Similarly, Jones oxidation of austalide B (**2**) gave the ketone (**19**) (12-H, δ_H 2.656 and 3.363, J 12.2 Hz). The ^{1}H and ^{13}C n.m.r. data of each of the above ketones [(**16**)—(**19**)] (see Experimental section) confirmed the structure of the corresponding alcohol. Base-catalysed hydrogen–deuterium exchange of these ketones resulted in the regio- and stereo-specific incorporation of deuterium.

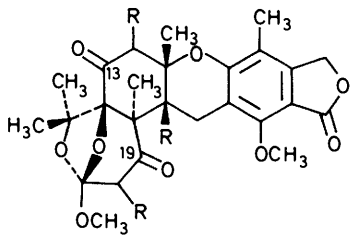
In a typical procedure, the monoketone (**17**), M^+ 488, was refluxed for 90 min in a solution of sodium methoxide in [*O*-^{2}H]methanol. Mass spectrometry of the product (**20**) gave the molecular ion at m/z 489, which corresponds to the incorporation of a single deuterium atom. It was evident from a comparison of the ^{1}H n.m.r. spectra of (**17**) and its deuteriated derivative (**20**) that complete exchange of one of the C-18 protons had occurred as the AX spin system (δ_H 2.724 and 2.956, J 15.8 Hz) in the spectrum of (**17**) had changed to a singlet resonance at δ_H 2.713.



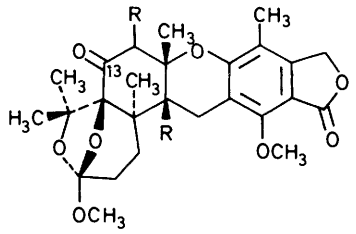
(**16**)



(**17**) R = H
(**20**) R = D



(**18**) R = H
(**21**) R = D



(**19**) R = H
(**22**) R = D

Mass spectrometry of the deuteriated diketone (**21**), M^+ 489 obtained by base-catalysed exchange of the diketone (**18**), M^+ 486, indicated that three deuterium atoms were incorporated. The ^{1}H n.m.r. spectrum of (**21**) displayed one-proton singlets for the C-12 (δ_H 2.790) and C-18 (δ_H 2.805) protons, which are consistent in each case with the stereospecific exchange of the methylene proton that resonates at lower field. The complete exchange of 21-H by deuterium is illustrated by the absence of the relevant doublet signal (δ_H 3.023, J 8.8 Hz) and the change of a double doublet at δ_H 2.758 (J 18.8 and 8.8 Hz), due to 22-H$_a$, to a doublet (J 18.9 Hz).

Base-catalysed deuterium exchange of the monoketone (**19**) yielded the dideuterioketone (**22**) and once again it was the C-12 methylene proton which resonates at lower field (δ_H 2.656) as well as the C-21 proton which were affected.

The ^{13}C n.m.r. data of the deuteriated ketones (**20**)—(**22**) confirm the deuterium distribution pattern in each compound. A comparison of the broad-band proton-decoupled ^{13}C n.m.r. spectra of the deuteriated and corresponding non-deuteriated compounds indicated small differences in the chemical shifts of the carbon atoms one and two bonds removed from a deuterium atom.[20–22] The magnitude of these deuterium isotope shifts can be of diagnostic use in structure determination and biosynthetic studies. The small amount of non-deuteriated material (**19**) present with (**22**) as indicated by the

^{13}C n.m.r. spectrum serves as an internal standard and allowed the accurate measurement of the deuterium isotope shifts. The presence of a single deuterium atom at C-12 in (**22**) is indicated by a triplet [J(CD) 19.3 Hz] in the broad-band proton-decoupled ^{13}C n.m.r. spectrum and the observed one-bond deuterium isotope shift (Δδ –0.30 p.p.m.) for this signal. A two-bond shift is observed for the C-11 (Δδ –0.08 p.p.m.) and C-13 (Δδ 0.05 p.p.m.) resonances. These two resonances appear as singlets in the proton-decoupled spectrum because (C,D) coupling over two bonds is negligible (*ca.* 1 Hz).[23] The exchange of 21-H in the ketone (**22**) by deuterium is evident from the upfield shift (Δδ –0.39 p.p.m.) of C-21 which gives rise to a triplet [J(CD) 20.5 Hz]. The C-20 and C-22 resonances were shifted upfield (Δδ –0.08 and –0.11 p.p.m., respectively) due to the presence of deuterium two-bonds removed. The observed one- and two-bond deuterium isotope shifts are of the same magnitude and sign (Δδ –0.34 to –0.44 p.p.m. and Δδ –0.08 to –0.11 p.p.m., respectively) as reported values.[20] The small downfield two-bond isotope shift (Δδ 0.05 p.p.m.) observed in the resonance position of C-13 in (**22**) is characteristic of carbonyl carbon atoms.[24] The magnitude of this shift is possibly an indication that the C-12 deuterium is situated in an axial (Δδ –0.06 p.p.m.) rather than an equatorial (Δδ –0.02 p.p.m.) position.[20] Similar isotope shifts were observed in the ^{13}C n.m.r. spectra of the deuteriated ketones (**20**) and (**21**).

A comparative structural analysis of these deuteriated compounds suggests that the abstraction of the 21-H proton is associated with the presence of the C-13 carbonyl group. The proposed mechanism for the exchange of 21-H, as well as the stereospecific exchange of a C-12 proton is shown in Figure 5 using the monoketone (**19**) as an example. The bathochromic shifts of 24 and 45 nm in the u.v. maxima at 220 and 266 nm, respectively for the ketone (**19**) on addition of sodium methoxide to the sample are indicative of the facile formation of the phenoxide anion.

The selective irradiation of the 21-H resonance in a ^1H-{^1H} n.O.e. experiment using the ketone (**18**), affected the 18-H signal (δ_H 3.143) which appears at lower field. This result taken in conjunction with the earlier observation that it is this C-18 proton which is selectively exchanged, assigns the 18*R* configuration to the deuteriated ketone (**21**). The chirality at C-12 in this compound could not be determined in a similar manner since the C-12 protons are obscured by the signals of the C-18 and C-28 protons.

The preferential exchange of the C-18 *pro-R* proton points to a chair-like conformation for the relevant six-membered ring in the transition state leading to the deuteriated derivative (**21**). This conformation, in which the C-18 *pro-R* proton is axial, allows efficient overlap of the C(18)–18-*pro-R*-H bond with the π-orbitals of the C-19 carbonyl bond. As a result, the transition state is stabilized [25] and the exchange of the *pro-R* proton is stereoelectronically assisted. The axial orientation of the C-18 *pro-R* proton is in agreement with the observation that it resonates at lower field than the C-18 *pro-S* proton, due to the anisotropy of the C-19 carbonyl group.[26] However, cognizance should be taken of possible steric influences not only on the chemical shift of the C-18 protons, but also on the stereochemical outcome of the reaction.[25]

The observed stereospecificity of exchange at C-12 in the ketones (**21**) and (**22**) is similarly ascribed to the nett result of the combined steric and stereoelectronic effects.

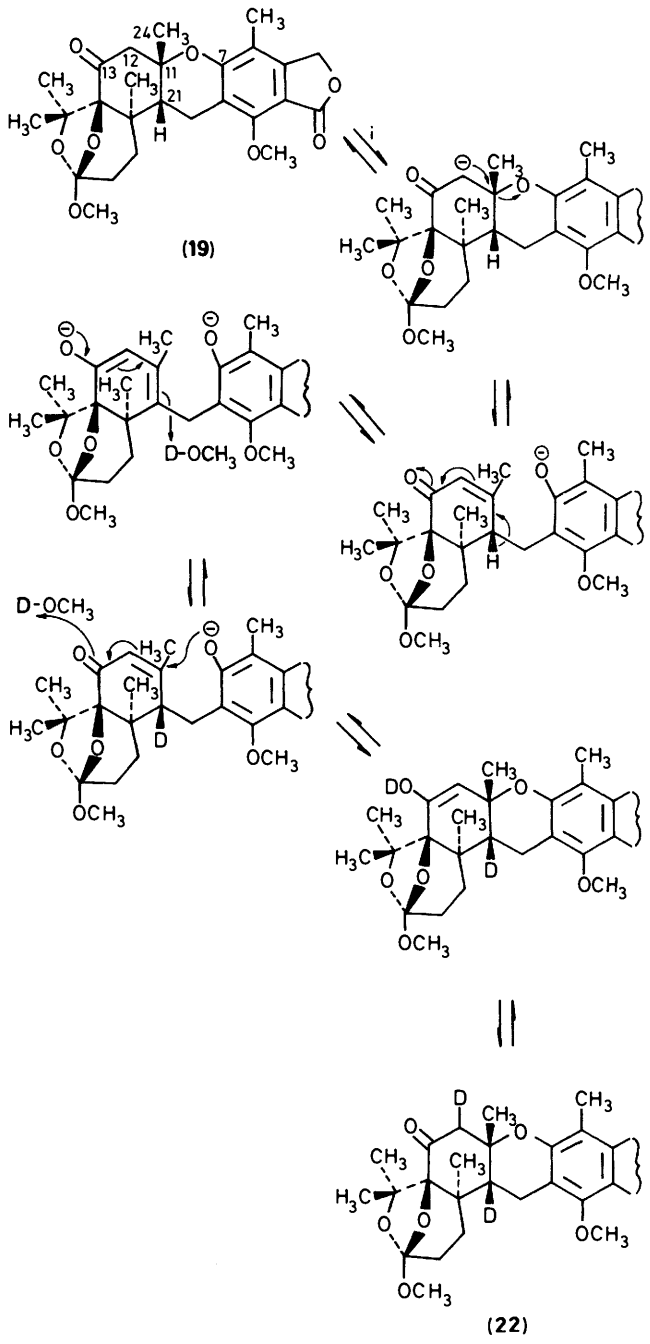


Figure 5. Proposed mechanism for deuterium incorporation into the monoketone (**19**). *Reagents:* (i) $NaOCH_3$–CH_3OD

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. U.v. absorptions were measured for solutions in methanol on a Unicam SP 8-100 spectrometer. I.r. spectra were recorded on a Perkin-Elmer 237 spectrometer for solutions in chloroform. Mass spectra were taken on an A.E.I. MS 9 or a Varian MAT 212 double focussing spectrometer. N.m.r. spectra of [^2H]chloroform solutions were recorded on a Bruker WM-500 spectrometer operating at 500.13 MHz for ^1H and 125.76 MHz for ^{13}C nuclei. Chemical shifts are reported in p.p.m. relative to tetramethylsilane (δ 0.000). The abbreviations s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad are used in connection with ^1H n.m.r. data. In the case of ^{13}C n.m.r. data, capital letters refer to the patterns resulting from directly bonded (C,H) couplings [^1J(CH)], unless otherwise stated. Optical rotations were measured at 24 °C on a

Perkin-Elmer 241 polarimeter for solutions in chloroform. Elemental analyses were performed by the Microanalytical Section of the NCRL.

Merck silica gel 60 (particle size 0.063—0.200 mm) or Merck silica gel 60 H for t.l.c., under pressure (150 kPa), and Merck aluminium oxide 90 (activity II–III, particle size 0.063—0.200 mm) were used for column chromatography.

Isolation of Austalides A—J.—Aspergillus ustus (MRC 1163)* was grown in bulk on wet, sterilized, whole yellow-maize kernels for 21 days at 25 °C. Cultures were dried at 45 °C for 24 h and milled to a fine meal. The resulting material was acutely toxic to day-old ducklings. The dried, milled, mouldy maize (16.5 kg) was extracted with chloroform–methanol (1:1 v/v) for 48 h. The solvent was removed under reduced pressure and the resulting, toxic extract was partitioned between aqueous methanol (90%) and n-hexane. The methanol solution was evaporated and the residual material partitioned between chloroform and water, yielding toxic material (267 g) in the organic layer. This material was fractionated on a formamide-impregnated cellulose powder column (3.0 kg) which was eluted with solvent of increased polarity from n-hexane, n-hexane–benzene, benzene, to benzene–ethyl acetate (1:1 v/v). Fractions (100 ml) were collected and combined on the basis of their chromatographic behaviour on t.l.c., affording five fractions (A—E). Each of these fractions was partitioned between chloroform and water to remove any traces of formamide. All five fractions were acutely toxic to day-old ducklings.

Fraction A. This fraction (165.0 g), highly lipidic in nature, was dissolved in aqueous methanol (90%) and repeatedly extracted with n-hexane. The resulting polar material (3.6 g) was subjected to column chromatography on silica gel 60H (500 g) with chloroform as eluant, to give, in order of elution, austocystin A (2.1 g),[4] *austalide A* (**1**) (260 mg), m.p. 212—214 °C (from chloroform–methanol), $[\alpha]_D$ −84.4° (*c* 1.00), $\lambda_{max.}$ 222 and 267 nm (ε 35 400 and 17 100, respectively), $\nu_{max.}$ 1 740 cm^{-1} (Found: C, 55.05; H, 5.85%; M^+, 516.236. $C_{28}H_{36}O_9 \cdot CHCl_3$ requires C, 54.77; H, 5.86%; *M*, 516.236), and 7-chloro-2-(3-furyl)-1,3,8-trimethoxyxanthone (110 mg).[8]

Fraction B. This fraction (25.0 g) was purified by silica gel column chromatography (2 kg) with chloroform as eluant to give, in order of elution, 4,6-*O*,*O*-bis-demethylaustocystin A (40 mg), 4-*O*-demethylaustocystin A (154 mg), 6-*O*-demethylaustocystin A (90 mg), 8-deoxy-6-*O*-methylversicolorin A (180 mg),[8] and a mixture (4.9 g). The mixture was subjected to column chromatography on silica gel with n-hexane–ethyl acetate (1:1 v/v) as eluant to give austocystin H (400 mg),[4] austalide A (**1**) (1.10 g), *austalide B* (**2**) (700 mg), m.p. 243—245 °C (from benzene–n-hexane), $[\alpha]_D$ −46.2° (*c* 1.00), $\lambda_{max.}$ 223 and 269 nm (ε 28 700 and 16 800, respectively), $\nu_{max.}$ 1 740 cm^{-1} (Found: C, 65.65; H, 7.2%; M^+, 474. $C_{26}H_{34}O_8$ requires C, 65.81; H, 7.22%; *M*, 474), δ_H 0.969 (3 H, s, 27-H), 1.249 (3 H, s, Me), 1.462 (3 H, s, Me), 1.631 (3 H, s, Me), 1.689—1.935 (4 H, m, 18- and 19-H), 2.022 (3 H, s, 23-H), 2.322 (2 H, d, *J* 3.3 Hz, 12-H), 2.379 (1 H, d, *J* 7.7 Hz, 21-H), 2.491 (1 H, d, *J* 8.5, 13-OH), 2.854 (1 H, dd, *J* 18.7 and 7.7 Hz, 22-H_b), 2.925 (1 H, d, *J* 18.7 Hz, 22-H_a), 3.397 (3 H, s, 28-H), 4.098 (1 H, dd, *J* 8.5 and 3.3 Hz, 13-H), 4.122 (3 H, s, 29-H), and 5.104 (2 H, s, 1-H); δ_C 10.65 Q (C-23), 18.53 T (C-22), 19.23 Q (C-27), 26.18 Q, 27.14 Q, 28.84 Q, 30.53 T, 31.54 T, 36.68 D (C-21), 39.74 S (C-20), 41.32 T (C-12), 48.75 Q (C-28), 62.01 Q (C-29), 68.15 T (C-1), 69.91 D (C-13), 78.29 S (C-11), 84.85 S, 86.35 S, 108.22 S, 114.26 S, 116.57 S, 118.95 S, 145.64 S, 155.43 S, 157.13, S, and 169.07 S (C-3); *austalide C* (**3**) (600 mg), as a white amorphous solid, $[\alpha]_D$ −99.0° (*c* 1.00), $\lambda_{max.}$ 221 and 265 nm (ε 26 900 and 13 800, respectively), $\nu_{max.}$ 1 740 cm^{-1} (Found: C, 62.45; H, 6.5%; M^+, 574.242. $C_{30}H_{38}O_{11}$ requires C, 62.7; H, 6.6%; *M*, 574.241); δ_H 1.023 (3 H, s, 27-H), 1.232 (3 H, s, Me), 1.348 (3 H, s, Me), 1.705 (3 H, s, Me), 1.951 (1 H, d, *J* 15.0 Hz, 18-H_b), 1.966 (3 H, s, 34-H), 2.034 (3 H, s, Me), 2.043 (3 H, s, 32-H), 2.147 (1 H, dd, *J* 16.0 and 4.2 Hz, 13-H_b), 2.202 (1 H, d, *J* 8.5 Hz, 21-H), 2.302 (1 H, dd, *J* 15.0 and 6.1 Hz, 18-H_a), 2.600 (1 H, dd, *J* 16.0 and 2.1 Hz, 12-H_a), 2.979 (1 H, dd, *J* 19.0 and 8.5 Hz, 22-H_b), 3.266 (1 H, d, *J* 19.0 Hz, 22-H_a), 3.414 (3 H, s, 28-H), 4.142 (3 H, s, 29-H), 5.077 (2 H, s, 1-H), 5.147 (1 H, dd, *J* 4.2 and 2.1 Hz, 13-H), and 5.506 (1 H, d, *J* 6.1 Hz, 19-H); δ_C 10.38 Q (C-23), 12.94 Q (C-27), 19.35 T (C-22), 21.02 Q (C-34), 21.06 Q, 25.44 Q, 27.63 Q, 29.37 Q, 37.12 T, 37.61 D (C-21), 37.83 T, 45.50 S (C-20), 48.79 Q (C-28), 62.04 Q (C-29), 68.05 T (C-1), 70.72 D, 70.95 D, 75.47 S (C-11), 85.25 S, 85.33 S, 107.76 S, 113.67 S, 115.34 S, 117.83 S (C-17), 145.61 S, 155.50 S, 157.36 S, 169.04 S, 169.22 S (C-33), and 170.06 S (C-31); and *austalide D* (**4**) (660 mg), m.p. 259—261 °C (from acetone), $[\alpha]_D$ −73.4° (*c* 1.00), $\lambda_{max.}$ 222 and 268 nm (ε 32 700 and 17 700, respectively); $\nu_{max.}$ 1 745 cm^{-1} (Found: C, 63.15; H, 6.8%; M^+, 532. $C_{28}H_{36}O_{10}$ requires C, 63.14; H, 6.81%; *M*, 532).

Fraction C. This fraction (11.9 g) was purified by silica gel column chromatography (1 kg); elution with n-hexane–ethyl acetate (3:2 v/v) gave in order of elution, 8-deoxy-6-*O*-methylversicolorin A (6 mg), austocystins A (20 mg), B (520 mg), and H (30 mg), and *austalides* D (**4**) (1.3 g) and *E* (**5**) (890 mg), m.p. 262—264 °C (from acetone), $[\alpha]_D$ −123.6° (*c* 1.00), $\lambda_{max.}$ 222 and 266 nm (ε 34 600 and 19 600, respectively), $\nu_{max.}$ 1 745 cm^{-1} (Found: C, 63.05, H, 6.85%; M^+, 532. $C_{28}H_{36}O_{10}$ requires C, 63.14; H, 6.18%; *M*, 532), δ_H 1.162 (3 H, s, 27-H), 1.200 (3 H, s, Me), 1.357 (3 H, s, Me), 1.690 (3 H, s, Me), 1.976 (3 H, s, 34-H), 2.043 (1 H, d, *J* 14.6 Hz, 18-H_b), 2.058 (3 H, s, Me), 2.108 (1 H, d, *J* 9.1 Hz, 21-H), 2.142 (1 H, dd, *J* 16.0 and 3.5 Hz, 12-H_b), 2.355 (1 H, dd, *J* 14.6 and 5.0 Hz, 18-H_a), 2.558 (1 H, br d, *J* 16.0 Hz, 12-H_a), 2.873 (1 H, dd, *J* 18.8 and 9.1 Hz, 22-H_b), 3.009 (1 H, d, *J* 18.8 Hz, 22-H_a), 3.410 (3 H, s, 28-H), 4.020 (1 H, m, 19-H), 4.105 (3 H, s, 29-H), 5.092 (2 H, s, 1-H), and 5.115 (1 H, m, 13-H); δ_C 10.38 Q (C-23), 13.34 Q (C-27), 19.10 T (C-22), 21.09 Q (C-34), 25.89 Q, 27.63 Q, 29.20 Q, 37.61 T (C-12), 37.75 D (C-21), 39.03 T (C-18), 46.29 S (C-20), 49.17 Q (C-28), 62.00 Q (C-29), 68.08 T (C-1), 70.76 D (C-19), 70.82 D (C-13), 75.53 S (C-11), 85.78 S, 86.03 S, 107.33 S, 113.66 S, 115.10 S, 118.76 S (C-17), 145.63 S, 155.33 S, 157.57 S, 169.13 S, and 169.36 S (C-33).

Subsequent elution of the column with n-hexane–ethyl acetate (3:7 v/v) gave *austalide F* (**6**) (40 mg), m.p. 261—263 °C (from acetone), $[\alpha]_D$ −57.7° (*c* 1.00), $\lambda_{max.}$ 220 and 264 nm (ε 30 900 and 16 800, respectively); $\nu_{max.}$ 1 740 cm^{-1} (Found: C, 63.65; H, 6.9%; M^+, 490. $C_{26}H_{34}O_9$ requires C, 63.66; H, 6.99%; *M*, 490); δ_H 1.144 (3 H, s, 27-H), 1.240 (3 H, s, Me), 1.491 (3 H, s, Me), 1.764 (3 H, s, Me), 2.034 (3 H, s, Me), 2.067—2.096 (2 H, m, 18-H), 2.313—2.422 (3 H, m), 2.710 (1 H, d, *J* 8.7 Hz, OH), 2.895 (1 H, dd, *J* 18.8 and 8.3 Hz, 22-H_b), 2.988 (1 H, d, *J* 18.8 Hz, 22-H_a), 3.051 (1 H, d, *J* 10.1 Hz, OH), 3.393 (3 H, s, 28-H), 3.922 (1 H, m, 19-H), 4.126 (3 H, s, 29-H), 4.131 (1 H, m, 13-H), and 5.110 (2 H, s, 1-H); δ_C 10.54 Q (C-23), 14.32 Q (C-27), 19.47 T (C-22), 26.02 Q, 27.23 Q, 29.36 Q, 38.14 T (C-18), 39.06 D (C-21), 40.78 T (C-12), 45.60 S (C-20), 49.09 Q (C-28), 62.05 Q (C-29), 68.07 T (C-1), 69.72 (C-13), 71.11 D (C-19), 78.31 S (C-11), 86.12 S, 86.93 S, 108.16 S, 114.08 S, 116.02 S, 118.40 S (C-17), 145.72 S, 155.35 S, 156.71 S, and 168.96 S (C-3); *austalide G* (97 mg) as a colourless glass, $[\alpha]_D$ −100.2° (*c* 1.00), $\lambda_{max.}$ 221 and 267 nm (ε 26 500 and 14 100, respectively), $\nu_{max.}$ 1 720—1770 cm^{-1} (Found: M^+, 518.251. $C_{28}H_{38}O_9$ requires *M*, 518.252), δ_H 1.022 (3 H, s, 27-H), 1.171 (3 H, s, Me), 1.380 (3 H, s, Me), 1.593 (1 H, s br, OH), 1.626 (1 H, d, *J* 8.2 Hz, 21-H), 1.697 (1 H, d, *J* 2.2 Hz, 14-H), 1.767 (1 H, dd, *J* 16.0 and 4.2 Hz, 12-H_b), 1.823 (1 H, ddd, *J* 15.0, 11.5 and 6.0 Hz, 19-H_b), 1.967 (3 H, s, Me), 1.968 (3 H, s, Me), 2.029 (3 H, s, Me), 2.250 (1 H, ddd, *J* 15.3, 11.2, and 6.0

* Single-spored freeze-dried cultures have been deposited in the culture collection of the South African Medical Research Council.

Hz, 18-H_b), 2.417 (1 H, ddd, *J* 15.0, 11.2, and 3.6 Hz, 19-H_a), 2.538 (1 H, ddd, *J* 15.3, 11.5, and 3.6 Hz, 18-H_a), 2.718 (1 H, dd, *J* 18.6 and 8.2 Hz, 22-H_b), 2.624 (1 H, dd, 16.0 and 2.3 Hz, 12-H_a), 3.004 (1 H, d, *J* 18.6 Hz, 22-H_a), 3.673 (3 H, s, 28-H), 4.111 (3 H, s, 29-H), 5.088 (2 H, s, 1-H), 5.391 (1 H, ddd, *J* 4.2, 2.3, and 2.2 Hz, 13-H); δ_C 10.53 Q (*J* 127.2 Hz, C-23), 17.24 T (*J* 130.2 Hz, C-22), 20.66 Q (*J* 126.1 Hz, C-27), 21.54 Q (*J* 129.1 Hz, C-34), 27.81 Q (*J* 126.6 Hz), 29.21 T (*J* 127.2 Hz, C-19), 29.80 Q (*J* 124.3 Hz), 34.26 Q (*J* 124.5 Hz), 34.50 T (*J* 128.0 Hz, C-18), 41.01 D (*J* 124.1 Hz, C-21), 41.30 S (C-20), 42.01 T (*J* 124.2 Hz, C-12), 51.67 D (*J* 126.2 Hz, C-14), 51.67 Q (*J* 146.6 Hz, C-28), 62.04 Q (*J* 145.1 Hz, C-29), 68.15 T (*J* 151.2 Hz, C-1), 75.15 S, 71.03 D (*J* 148.5 Hz, C-13), 75.56 S, 107.38 S, 113.79 S, 115.36 S, 145.58 S, 155.46 S, 158.10 S, 169.27 S, 170.29 S, and 174.47 S (C-17); *austalide I* (470 mg), m.p. 236—238 °C (from n-hexane–acetone), $[\alpha]_D$ −132.6° (*c* 1.00), $\lambda_{max.}$ 221 and 266 nm (35 600 and 17 000, respectively); $\nu_{max.}$ 1 720—1 750 cm^{-1} (Found: C, 66.4; H, 6.8; M^+, 486.225. $C_{27}H_{34}O_8$ requires C, 66.65; H, 7.04%; *M*, 486.225); δ_H 1.065 (3 H, s, 27-H), 1.205 (3 H, s, Me), 1.486 (3 H, s, Me), 1.501 (3 H, s, Me), 1.571 (1 H, d, *J* 8.1 Hz, 21-H), 1.829 (1 H, m), 1.862 (1 H, d, *J* 3.9 Hz, 14-H), 1.989 (3 H, s, Me), 2.005 (3 H, s, Me), 2.085—2.135 (1 H, m), 2.677—2.726 (2 H, m), 2.783—2.848 (2 H, m), 2.844 (1 H, dd, *J* 18.8 and 8.1 Hz, 22-H_b), 3.079 (1 H, d, *J* 18.8 Hz, 22-H_a), 4.127 (3 H, s, 29-H), 5.103 (2 H, s, 1-H), and 5.407 (1 H, m, 13-H); δ_C 10.23 Q (*J* 128.0 Hz, C-23), 15.84 Q (*J* 126.7 Hz, C-27), 18.10 T (*J* 130.1 Hz, C-22), 21.23 Q (*J* 129.2 Hz, C-34), 26.48 Q (*J* 127.4 Hz), 27.43 Q (*J* 127.0 Hz), 31.24 T (*J* 129.3 Hz, C-19), 33.89 Q (*J* 129.1 Hz), 36.59 T (*J* 127.3 Hz, C-18), 40.86 S (C-20), 41.63 T (*J* 127.0 Hz, C-12), 45.94 D (*J* 125.4 Hz, C-21), 55.35 D (*J* 120.6 Hz, C-14), 61.86 Q (*J* 145.2 Hz, C-29), 67.99 T (*J* 151.5 Hz, C-1), 69.92 D (*J* 151.0 Hz, C-13), 74.96 S (C-11), 85.12 S (C-15), 107.30 S, 113.74 S, 114.82 S, 145.56 S, 155.14 S, 157.58 S, 168.97 S, 169.76 S, and 174.40 S (C-17); and *austalide J* (60 mg), m.p. 284—286 °C (from methanol), $[\alpha]_D$ −42.1° (*c* 1.00), $\lambda_{max.}$ 221 and 267 nm (ε 31 900 and 16 200, respectively), $\nu_{max.}$ 1 720, 1 750, and 1 760 cm^{-1} (Found: C, 67.75; H, 7.35%; M^+, 444.214. $C_{25}H_{32}O_7$ requires C, 67.55; H, 7.26%; *M*, 444.213); δ_H 0.852 (3 H, s, 27-H), 1.190 (3 H, s, Me), 1.365 (3 H, s, Me), 1.385 (3 H, s, Me), 1.719—1.748 (1 H, m), 1.826—1.900 (1 H, m), 1.925—1.965 (2 H, m), 2.020 (3 H, s, 23-H), 2.035—2.176 (2 H, m), 2.417—2.517 (2 H, m), 2.620—2.683 (1 H, m), 2.799 (1 H, dd, *J* 18.6 and 8.0 Hz, 22-H_b), 2.909 (1 H, d, *J* 18.6 Hz, 22-H_a), 4.091 (3 H, s, 29-H), and 5.090 (2 H, s, 1-H); δ_C 10.57 Q (*J* 128.1 Hz, C-23), 17.94 T (*J* 130.1 Hz, C-22), 20.75 Q (*J* 127.0 Hz, C-27), 25.22 T (*J* 127.1 Hz), 26.90 Q (*J* 127.0 Hz), 27.14 T (*J* 127.0 Hz), 29.13 T (*J* 127.1 Hz, C-19), 29.13 Q (*J* 127.1 Hz), 30.12 Q (*J* 126.9 Hz), 32.98 T (*J* 127.0 Hz), 37.43 D (*J* 125.2 Hz, C-21), 39.73 S (C-20), 61.93 Q (*J* 146.1 Hz, C-29), 68.18 T (*J* 150.9 Hz, C-1), 75.85 S (C-11), 79.58 S (C-15), 91.33 S (C-14), 107.38 S, 114.38 S, 115.26 S, 145.63 S, 155.18 S, 158.22 S, 169.27 S, and 173.38 S (C-17).

Fraction D. This fraction (21.3 g) was purified by silica-gel column chromatography (2 kg) with chloroform–methanol (98:2 v/v) as eluant to give, in order of elution, 8-deoxy-6-*O*-methylversicolorin A (20 mg), austocystin D (500 mg),[4] and a mixture (590 mg). This mixture was subjected to column chromatography on silica gel 60H (150 g) with n-hexane–ethyl acetate (1:1 v/v) as eluant to afford *austalide F* (**5**) (20 mg), *G* (30 mg), and *H* (50 mg) as a colourless glass, $[\alpha]_D$ −19.5° (*c* 1.00), $\lambda_{max.}$ 221 and 267 nm (ε 20 400 and 9 600, respectively), $\nu_{max.}$ 1 740 cm^{-1} (Found: M^+, 476.240. $C_{26}H_{36}O_8$ requires *M*, 476.241); δ_H 0.962 (3 H, s, 27-H), 1.244 (3 H, s, Me), 1.322 (1 H, d, *J* 2.5 Hz, 14-H), 1.431 (3 H, s, Me), 1.437 (3 H, s, Me), 1.655 (1 H, d, *J* 7.9 Hz, 21-H), 1.799 (1 H, dd, *J* 15.3 and 3.7 Hz, 12-H_b), 1.885—1.998 (1 H, m), 2.022 (3 H, s, 23-H), 2.187-2.273 (2 H, m), 2.291—2.389 (1 H, m), 2.504 (1 H, dd, *J* 15.3 and 3.1 Hz, 12-H_a), 2.771 (1 H, dd, *J* 18.7 and 7.9 Hz, 22-H_b), 3.071 (1 H, *J* 18.7 Hz, 22-H_a), 3.683 (3 H, s, 28-H), 4.128 (3 H, s, 29-H), 4.629 (1 H, m, 13-H), and 5.100 (2 H, s, 1-H); δ_C 10.76 Q (*J* 128.1 Hz, C-23), 18.49 T (*J* 127.9 Hz, C-22), 21.48 Q (*J* 126.2 Hz, C-27), 27.44 Q (*J* 126.8 Hz), 29.62 T (*J* 124.0 Hz), 31.67 Q (*J* 124.5 Hz), 33.49 Q (*J* 124.8 Hz), 35.00 T (*J* 124.4 Hz), 40.41 D (*J* 126.9 Hz, C-21), 40.96 S (C-20), 43.99 T (*J* 126.7 Hz, C-12), 49.43 D (*J* 116.8 Hz, C-14), 51.82 Q (*J* 146.5 Hz, C-28), 62.17 Q (*J* 144.8 Hz, C-29), 68.15 T (*J* 151.2 Hz, C-1), 69.96 D (*J* 148.4 Hz, C-13), 75.61 S, 78.60 S, 108.51 S, 114.09 S, 116.00 S, 145.78 S, 155.51 S, 156.50 S, 160.01 S, (C-3), and 173.87 S (C-17).

Fraction E. This fraction (31.5 g) was purified by preparative h.p.l.c. on a silica gel column. Elution with chloroform–methanol (98:2 v/v) yielded austocystin D (5.1 g) and a mixture of orange-red pigments (4.9 g). The pigments were separated on silica gel (500 g) impregnated with oxalic acid. Elution withn-hexane–acetone (7:3 v/v) yielded averufanin (60 mg), averuin (140 mg), and versicolorin C (150 mg).[8]

Isolation of Austalides K and L.—Ten aliquots (100 ml) of a malt extract medium (150 g/l) in Erlenmeyer flasks (500 ml) were inoculated with spores of *Aspergillus ustus* (MRC 1163) obtained from one-week potato–dextrose–agar slants, and incubated at 25 °C in static culture. After 14 days the cultures were filtered and the mycelium macerated with acetone in a Waring blender. The acetone mixture was filtered, evaporated, and the resulting brown gum was partitioned between aqueous methanol (90%) and n-hexane. Evaporation of the methanol solution gave a residue which was partitioned between chloroform and water. The organic layer was concentrated and percolated through an aluminium oxide column (50 g) with chloroform–methanol (95:5 v/v; 200 ml). The combined eluants were evaporated to give a light-yellow gum (200 mg). This material was subjected to column chromatography on silica gel (100 g) with n-hexane–ethyl acetate (1:1 v/v) as eluant. Fractions (7.0 ml) were evaluated by t.l.c. (n-hexane–ethyl acetate, 1:1 v/v) and appropriate fractions combined to give three fractions: R_F 0.48 (81 mg), R_F 0.31 (73 mg), and R_F 0.13 (27 mg). The latter fraction crystallized from n-hexane–acetone to give a compound (22 mg), identical with an authentic sample of austalide J. The material with R_F 0.48 was purified on a silica gel column (20 g). Elution with chloroform–methanol (99:1 v/v) gave austalide A (**1**) (9 mg), identical with an authentic sample, and *austalide K* (42 mg), as a white glass, $[\alpha]_D$ −75.9° (*c* 1.00), $\lambda_{max.}$ 222 and 267 nm (ε 18 900 and 11 000, respectively), $\nu_{max.}$ 1 700 and 1 745 cm^{-1} (Found: C, 72.6; H, 7.9%; M^+, 412.224. $C_{25}H_{32}O_5$ requires C, 72.79; H, 7.82%; *M*, 412.225), δ_H 0.697 (3 H, s, 27-H), 0.999 (3 H, s, Me), 1.090 (3 H, s, Me), 1.165 (3 H, s, Me), 1.458—1.518 (4 H, m, 13-H_b, 14-H, 19-H_b, and 21-H), 1.615 (1 H, ddd, *J* 13.9, 13.9, and 4.2 Hz, 12-H_b), 1.785 (1 H, dddd, *J* 13.9, 13.9, 13.9, and 3.0 Hz, 13-H_a), 2.018 (3 H, s, 23-H), 2.072 (1 H, ddd, 13.3, 6.9, and 3.8 Hz, 19-H_a), 2.260 (1 H, ddd *J* 13.9, 3.0, and 3.0 Hz, 12-H_a), 2.365 (1 H, ddd, *J* 16.1, 6.5, and 3.8 Hz, 18-H_b), 2.485 (1 H, ddd, *J* 16.1, 11.6, and 6.9 Hz, 18-H_a), 2.790 (1 H, dd, *J* 18.5 and 8.0 Hz, 22-H_b), 2.910 (1 H, d, *J* 18.5 Hz, 22-H_a), 4.078 (3 H, s, 29-H), and 5.087 (2 H, s, 1-H); δ_C 10.51 Q (*J* 128.2 Hz, C-23), 14.07 Q (*J* 125.1 Hz, C-27), 18.15 T (*J* 130.3 Hz, C-22), 21.53 Q (*J* 126.9 Hz), 26.61 Q (*J* 127.0 Hz), 26.97 Q (*J* 127.1 Hz), 29.54 T (*J* 125.5 Hz), 33.91 T (*J* 130.0 Hz, C-18), 37.49 S (C-20), 38.24 T (*J* 127.8 Hz), 39.60 T (*J* 124.5 Hz, C-19), 46.95 D (*J* 125.5 Hz, C-21), 47.09 S (C-15), 54.07 D (*J* 120.9 Hz, C-14), 61.75 Q (*J* 145.4 Hz, C-29), 68.07 T (*J* 150.9 Hz, C-1), 76.20 S (C-11), 107.18 S, 114.29 S, 115.14 S, 145.38 S, 155.23 S, 158.45 S, 169.17 S, (C-3), and 216.23 S (C-17).

The material with R_F 0.31 was purified on a silica gel column (20 g). Elution with chloroform–methanol (99:1 v/v) gave austalide D (**4**) (10 mg), identical with an authentic sample, and *austalide L* (40 mg), m.p. 207—208 °C (from benzene–n-hexane), $[\alpha]_D$ −71.0° (*c* 1.00), $\lambda_{max.}$ 223 and 269 nm (ε 31 200 and 16 600, respectively), $\nu_{max.}$ 1 700 and 1 745 cm^{-1} (Found: C, 69.8, H, 7.4%, M^+, 428.219. $C_{25}H_{32}O_6$ requires C, 70.07; H,

7.53%, *M*, 428.220); δ_H 0.780 (3 H, s, 27-H), 1.092 (3 H, s, Me), 1.128 (3 H, s, Me), 1.167 (3 H, s, Me), 1.467 (1 H, ddd, *J* 13.4, 4.0, and 4.0 Hz, 12-H_b), 1.751 (1 H, ddd, *J* 12.9, 8.0, and 4.0 Hz, 19-H_b), 1.979—2.038 (2 H, m, 13-H_b and 19-H_a), 2.015 (3 H, s, 23-*H*), 2.075 (*1* H, ddd, *J* 13.4, 13.4, and 4.0 Hz, 13-H_a), 2.155 (1 H, ddd, 13.4, 13.4, and 4.0 Hz, 12-H_a), 2.254 (1 H, dd, *J* 7.0 and 1.9 Hz, 21-H), 2.463 (1 H, ddd, *J* 15.6, 8.0, and 8.0 Hz, 18-H_b), 2.549 (1 H, ddd, *J* 15.6, 8.0, and 4.0 Hz, 18-H_a), 2.740—2.831 (2 H, m, 22-H), 4.061 (3 H, s, 29-H), and 5.078 (2 H, s, 1-H); δ_C 10.61 Q (*J* 128.1 Hz, C-23), 18.04 T (*J* 130.2 Hz, C-22), 18.28 Q (*J* 124.9 Hz, C-27), 21.64 Q (*J* 128.0 Hz), 23.59 Q (*J* 126.9 Hz), 24.19 T (*J* 126.3 Hz), 26.81 Q (*J* 126.4 Hz), 33.16 T (*J* 128.9 Hz, C-19), 33.39 T (*J* 129.6 Hz), 33.79 T (*J* 126.8 Hz, C-18), 40.73 D (*J* 129.5 Hz, C-21), 41.23 S (C-20), 52.62 S (C-15), 61.83 Q (*J* 145.6, Hz, C-29), 68.18 T (*J* 150.9 Hz, C-1), 76.14 S (C-11), 79.53 S (C-14), 107.23 S, 114.40 S, 115.84 S, 145.41 S, 155.29 S, 158.59 S, 169.35 S (C-3), and 216.40 S (C-17).

Alkaline Hydrolysis of Austalide C (**3**).—Austalide C (30 mg) was treated with 0.1M-KOH in methanol for 16 h at room temperature. The reaction mixture was acidified (1M-HCl), diluted with water (50 ml), and extracted with chloroform (3 × 50 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give an alcohol (21 mg, 82%), identical with an authentic sample of austalide F (**6**).

Alkaline Hydrolysis of Austalide D (**4**).—Austalide D (100 mg) was hydrolysed as above to give a diol (88 mg, 96%) identical with an authentic sample of austalide F (**6**).

Acetylation of Austalide E (**5**).—Austalide E (20 mg) in pyridine (5 ml) was refluxed with acetic anhydride (0.5 ml) for 16 h (t.l.c. control). The cooled reaction mixture was stirred with crushed ice for 1 h and extracted with benzene (3 × 30 ml). The combined benzene fractions were washed with 0.1M-HCl (3 × 50 ml), water (3 × 50 ml), and dried ($MgSO_4$). The benzene was evaporated under reduced pressure to give a gum which was purified by column chromatography on silica gel (20 g) with n-hexane–ethyl acetate (1:1 v/v) as eluant. A single compound (17 mg, 79%) was obtained, identical with an authentic sample of austalide C (**3**).

Alkaline Hydrolysis of Austalide E (**5**).—Austalide E (150 mg) was hydrolysed as above to give a diol (130 mg, 94%), identical with an authentic sample of austalide F (**6**).

Alkaline Hydrolysis of Austalide A (**1**).—Austalide A (100 mg) was hydrolysed as above to give the alcohol (80 mg, 74%), identical with an authentic sample of austalide B (**2**).

Lithium Aluminium Hydride Reduction of Austalide D (**4**).—Austalide D (532 mg) in dry tetrahydrofuran was stirred with a suspension of lithium aluminium hydride (200 mg) in tetrahydrofuran (100 ml). The mixture was refluxed for 20 h, cooled, and the excess of reagent decomposed by slow addition of water. The mixture was acidified (1M-HCl), extracted with chloroform, and dried ($MgSO_4$). The solvent was removed to give a gum (397 mg) which was purified on a silica-gel column (75 g). Elution with chloroform–methanol (95:5 v/v) afforded austalide F (**6**) (40 mg, 8%), identical with an authentic sample, and the *alcohol* (**14**) (286 mg, 58%) as a colourless glass, $[\alpha]_D$ −18.5° (*c* 1.00), $\lambda_{max.}$ 228, 281, and 288 nm (ε 11 700, 6 900, and 7 300, respectively) (Found: M^+, 494.252. $C_{26}H_{38}O_9$ requires *M*, 494.252); δ_H 1.146 (3 H, s, Me), 1.236 (3 H, s, Me), 1.486 (3 H, s, Me), 1.759 (3 H, s, Me), 2.039 (1 H, d, *J* 7.5 Hz, 21-H), 2.073 (1 H, d, *J* 14.4 Hz, 12-H_b), 2.207 (3 H, s, 23-H), 2.278 (*1* H, dd, *J* 15.7 and 4.0 Hz, 18-H_b), 2.332 (1 H, dd, *J* 15.7 and 3.6 Hz, 18-H_a), 2.335 (1 H, dd, *J* 14.4 and 5.0 Hz, 12-H_a), 2.903 (1 H, dd, *J* 18.6 and 7.5 Hz, 22-H_b), 2.958 (1 H, d, *J* 18.6 Hz, 22-H_a), 3.014 (1 H, d, *J* 9.1 Hz, OH), 3.072 (1 H, d, *J* 10.0 Hz, OH), 3.392 (3 H, s, 28-H), 2.766 (3 H, s, 29-H), 3.895—3.923 (1 H, m, 19-H), 4.108—4.139 (1 H, m, 13-H), 4.763 (2 H, s, 1-H), 4.782 (1 H, d, *J* 11.7 Hz, 3-H_b), and 4.824 (1 H, d, *J* 11.7 Hz, 3-H_a); δ_C 10.89 Q (*J* 128.0 Hz, C-23), 14.22 Q (*J* 126.7 Hz, C-27), 19.46 T (*J* 128.0 Hz, C-22), 25.95 Q (*J* 126.5 Hz), 26.98 Q (*J* 126.5 Hz), 29.29 Q (*J* 126.3 Hz), 38.48 D (*J* 129.5 Hz, C-21), 39.05 T (*J* 130.1 Hz, C-18), 40.65 T (*J* 128.6 Hz, C-12), 45.47 S (C-20), 48.94 Q (*J* 143.4 Hz, C-28), 56.45 T (*J* 143.0 Hz, C-3), 58.80 T (*J* 142.5 Hz, C-1), 61.55 Q (*J* 144.1 Hz, C-29), 69.79 D (*J* 147.4 Hz), 70.89 D (*J* 145.4 Hz), 77.03 S (C-11), 86.02 S, 86.94 S, 115.76 S, 118.13 S (C-17), 121.51 S, 125.22 S, 137.61 S, 151.15 S, and 154.02 S.

Barium Manganate Oxidation of the Alcohol (**14**).—The alcohol (**14**) (100 mg) in chloroform was stirred with barium manganate (300 mg) for 24 h at room temperature. Filtration and evaporation of the solvent yielded a gum (82 mg) which was purified on a silica gel column (34 g). Elution with n-hexane–ethyl acetate (1:1 v/v) gave austalide F (**6**) (11 mg, 11%) identical with an authentic sample, and the *isomer* (**15**) (13 mg, 13%), as a white amorphous solid, $[\alpha]_D$ −42.9° (*c* 1.00), $\lambda_{max.}$ 217, 256, and 310 nm (ε 25 600, 6 000, and 2 800, respectively), $\nu_{max.}$ 1 745 and 1 760 cm^{-1} (Found: M^+, 490.219. $C_{26}H_{34}O_9$ requires *M*, 490.219), δ_H 1.146 (3 H, s, Me), 1.217 (3 H, s, Me), 1.494 (3 H, s, Me), 1.764 (3 H, s, Me), 2.062—2.093 (2 H, m, 18-H_b and 21-H), 2.277—2.375 (3 H, m, 12-H and 18-H_a), 2.448 (3 H, s, 23-H), 2.874 (1 H, dd, *J* 19.4 and 8.4 Hz, 22-H_b), 2.875 (1 H, d, *J* 9.2 Hz, OH), 2.977 (1 H, d, *J* 19.4 Hz, 22-H_a), 3.079 (1 H, d, *J* 9.3 Hz, OH), 3.393 (3 H, s, 28-H), 3.893—3.937 (1 H, m, 19-H), 3.902 (3 H, s, 29-H), 4.122—4.145 (1 H, m, 13-H), 5.319 (1 H, d, *J* 14.3 Hz, 3-H_b), and 5.372 (1 H, d, *J* 14.3 Hz, 3-H_a).

Jones Oxidation of Austalide D (**4**).—Austalide D (212 mg), in acetone (20 ml) was stirred with Jones reagent (10 ml) for 1 h at room temperature. The reaction mixture was diluted with cold water (100 ml) and extracted with chloroform (3 × 50 ml). The combined chloroform extracts were washed with water (3 × 50 ml), dried ($MgSO_4$), and evaporated under reduced pressure to give a white amorphous solid. Crystallization of this material from benzene–n-hexane gave the *ketone* (**16**) (200 mg, 95%) as white plates, m.p. 124—126 °C, $[\alpha]_D$ −78.3° (*c* 0.90), $\lambda_{max.}$ 220 and 264 nm (ε 28 900 and 12 200, respectively), $\nu_{max.}$ 1 740br cm^{-1} (Found: M^+, 530.214. $C_{28}H_{34}O_{10}$ requires *M*, 530.215); δ_H 0.668 (3 H, s, 27-H), 1.368 (3 H, s, Me), 1.385 (3 H, s, Me), 1.694 (3 H, s, Me), 2.013 (3 H, s, Me), 2.027 (1 H, d, *J* 15.2 Hz, 18-H_b), 2.036 (3 H, s, Me), 2.632 (1 H, d, *J* 8.5 Hz, 21-H), 2.649 (1 H, dd, *J* 15.2 and 6.0 Hz, 18-H_a), 2.683 (1 H, d, *J* 12.0 Hz, 12-H_b), 3.002 (1 H, dd, *J* 18.9 and 8.5 Hz, 22-H_b), 3.164 (1 H, d, *J* 18.9 Hz, 22-H_a), 3.434 (3 H, s, 28-H), 3.442 (1 H, d, *J* 12.0 Hz, 12-H_a), 4.127 (3 H, s, 29-H), 5.077 (2 H, s, 1-H), and 5.480 (1 H, d, *J* 6.0 Hz, 19-H); δ_C 10.42 Q (C-23), 11.13 Q (C-27), 19.46 T (C-22), 20.98 Q (C-32), 24.31 Q, 27.26 Q, 30.98 Q, 37.01 T (C-18), 38.49 D (C-21), 48.94 Q (C-28), 50.79 T (C-12), 51.37 S (C-20), 62.15 Q (C-29), 68.12 T (C-1), 69.56 D (C-19), 81.43 S (C-11), 83.37 S (C-15), 92.79 S (C-14), 108.16 S, 114.32 S, 114.52 S, 117.06 S (C-17), 145.79 S, 155.35 S, 156.79 S, 168.94 S (C-3), 169.94 S (C-31), and 203.70 S (C-13).

Jones Oxidation of Austalide F (**6**).—Austalide F (130 mg) in acetone (20 ml) was treated with Jones reagent (0.5 ml) for 10 min at room temperature. Work-up as before (see above), gave a mixture of two products which were separated by chromatography on silica gel (15 g). Elution with n-hexane–ethyl

acetate (1:1 v/v) afforded the *diketone* (**18**) (28 mg, 22%), a white solid, $[\alpha]_D$ −66.1° (*c* 1.00), $\lambda_{max.}$ 222 and 268 nm (ε 20 800 and 10 100, respectively), $\nu_{max.}$ 1 730br cm^{-1} (Found: M^+, 486.187. $C_{26}H_{30}O_9$ requires *M*, 486.189); δ_H 0.818 (3 H, s, 27-H), 1.242 (3 H, s, Me), 1.362 (3 H, s, Me), 1.372 (3 H, s, Me), 2.001 (3 H, s, Me), 2.478 (1 H, d, *J* 18.8 Hz, 22-H_b), 2.758 (1 H, dd, *J* 18.8 and 8.8 Hz, 22-H_a), 2.805 (1 H, d, *J* 12.7 Hz, 12-H_b), 2.808 (1 H, d, *J* 15.8 Hz, 18-H_b), 3.023 (1 H, d, *J* 8.8 Hz, 21-H), 3.143 (1 H, d, *J* 15.8 Hz, 18-H_a), 3.460 (1 H, d *J* 12.7 Hz, 12-H_a), 3.463 (3 H, s, 28-H), 4.061 (3 H, s, 29-H), 5.057 (1 H, *J* 15.1 Hz, 1-H_b), and 5.093 (1 H, d, *J* 15.1 Hz, 1-H_a); δ_C 10.35 Q (C-23), 10.51 Q (C-27), 17.65 T (C-22), 25.31 Q, 27.16 Q, 28.84 Q, 37.77 D (C-21), 48.85 T (C-18), 49.51 Q (C-28), 51.01 T (C-12), 60.98 S (C-20), 62.10 Q (C-29), 68.15 T (C-1), 81.76 S (C-11), 83.15 S (C-15), 91.11 S (C-14), 108.24 S, 114.06 S, 114.72 S, 117.89 S (C-17), 145.93 S, 155.39 S, 156.77 S, 168.89 S (C-3), 202.10 S (C-19), and 203.58 S (C-13); followed by the *monoketone* (**17**) (95 mg, 74%), a white solid, $[\alpha]_D$ −125.3° (*c* 1.00), $\lambda_{max.}$ 222 and 268 nm (ε 21 600 and 10 200, respectively), $\nu_{max.}$ 1 715 and 1 745 cm^{-1} (Found: M^+, 488.204. $C_{26}H_{32}O_9$ requires *M*, 488.205), δ_H 1.171 (3 H, s, Me), 1.250 (3 H, s, Me), 1.316 (3 H, s, Me), 1.454 (3 H, s, Me), 2.012 (3 H, s, 23-H), 2.398—2.473 (2 H, m, 12-H), 2.470 (1 H, d, *J* 8.4 Hz, 21-H), 2.552 (1 H, d, *J* 18.6 Hz, 22-H_b), 2.700 (1 H, dd, *J* 18.6 and 8.4 Hz, 22-H_a), 2.724 (1 H, d, *J* 15.8 Hz, 18-H_b), 2.956 (1 H, d, *J* 15.8 Hz, 18-H_a), 3.409 (3 H, s, 28-H), 4.066 (3 H, s, 29-H), 4.292 (1 H, s br, 13-H), and 5.088 (2 H, s, 1-H); δ_C 10.66 Q (C-23), 12.60 Q (C-27), 17.96 T (C-22), 27.03 Q, 27.15 Q, 27.17 Q, 37.16 D (C-21), 41.49 T (C-12), 48.36 T (C-18), 49.00 Q (C-28), 55.42 S (C-20), 62.03 Q (C-29), 68.13 T (C-1), 68.70 D (C-13), 76.32 S (C-11), 85.12 S, 85.35 S, 108.53 S, 114.36 S, 116.00 S, 117.57 S (C-17), 145.78 S, 155.51 S, 156.73 S, 168.93 S (C-3), and 207.12 S (C-19).

Jones Oxidation of Austalide B (**2**).—Austalide B (80 mg), in acetone (20 ml) was treated with Jones reagent (3.0 ml) for 1 h at room temperature. Work-up as before (see above), gave a crude product which was purified by chromatography on silica gel (15 g). Elution with n-hexane–ethyl acetate (1:1 v/v) gave the *ketone* (**19**) (42 mg, 53%), a white solid, $[\alpha]_D$ −79.2° (*c* 1.00), $\lambda_{max.}$ 220 and 266 nm (ε 30 600 and 14 600, respectively), $\nu_{max.}$ 1 740 cm^{-1} (Found: M^+, 472.208. $C_{26}H_{32}O_8$ requires *M*, 472, 210), δ_H 0.648 (3 H, s, 27-H), 1.349 (3 H, s, Me), 1.358 (3 H, s, Me), 1.527 (3 H, s, Me), 1.761—1.802 (1 H, m), 1.838—1.904 (1 H, m), 1.974—2.050 (2 H, m), 1.998 (3 H, s, 23-H), 2.656 (1 H, d, *J* 12.2 Hz, 12-H_b), 2.808—2.915 (3 H, m, 21- and 22-H), 3.363 (1 H, d, *J* 12.2 Hz, 12-H_a), 3.427 (3 H, s, 28-H), 4.101 (3 H, s, 29-H), and 5.077 (2 H, s, 1-H); δ_C 10.51 Q (C-23), 16.27 Q (C-27), 18.35 T (C-22), 24.55 Q, 27.14 Q, 30.10 T, 30.26 Q, 30.37 T, 36.70 D (C-21), 47.10 S (C-20), 49.03 Q (C-28), 51.36 T (C-12), 62.07 Q (C-29), 68.19 T (C-1), 82.23 S (C-11), 82.66 S (C-15), 93.44 S (C-14), 107.80 S, 114.66 S, 114.67 S, 118.58 S (C-17), 145.73 S, 155.27 S, 157.31 S, 169.12 S (C-3), and 204.02 S (C-13).

Deuteriation of the Monoketone (**17**).—The ketone (**17**) (40 mg) was dissolved in [*O*-^{2}H]methanol (99 atom% deuterium, 40 ml), a minimum quantity of sodium was added and the mixture was refluxed for 1.5 h. Acetyl chloride (1 ml) was added to deuterium oxide (1 ml) and 0.2 ml of this solution was added to the cooled reaction mixture. The resulting solution was diluted with chloroform (100 ml), washed with deuterium oxide (3 × 20 ml), dried ($MgSO_4$), and the solvent removed under reduced pressure to give the *monodeuterioketone* (**20**) (38 mg, 95%), as a white solid with t.l.c. behaviour identical with that of the starting material (Found: M^+, 489.210. $C_{26}H_{31}{}^2HO_9$ requires *M*, 489.211), δ_H 1.172 (3 H, s, Me), 1.250 (3 H, s, Me), 1.319 (3 H, s, Me), 1.455 (3 H, s, Me), 2.011 (3 H, s, 23-H), 2.398—2.470 (2 H, m, 12-H), 2.467 (1 H, d, *J* 8.4 Hz, 21-H), 2.554 (1 H, d, *J* 18.6 Hz, 22-H_b), 2.701 (1 H, dd, *J* 18.6 and 8.4 Hz, 22-H_a), 2.713 (1 H, s, 18-H_b), 3.411 (3 H, s, 28-H), 4.071 (3 H, s, 29-H), 4.292 (1 H, s br, 13-H), and 5.086 (2 H, s, 1-H); δ_C* 10.67 (C-23), 12.62 (C-27), 17.99 (C-22), 27.05, 27.16, 27.21, 37.15 (C-21), 41.51 (C-12), 48.08 T [*J* (CD) 19.2 Hz, C-18], 49.00 (C-28), 55.44 (C-20), 62.06 (C-29), 68.13 (C-1), 68.73 (C-13), 76.35 (C-11), 85.12, 85.36, 108.56, 114.35, 116.02, 117.57 (C-17), 145.79, 155.54, 156.72, 168.92 (C-3), and 207.12 (C-19).

Deuteriation of the Diketone (**18**).—The diketone (**18**) (28 mg) was deuteriated (see above), to give the *trideuterioketone* (**21**) (22 mg, 79%) as a white solid, with t.l.c. behaviour identical with that of the starting material (Found: M^+, 489.208. $C_{26}H_{27}{}^2H_3O_9$ requires *M*, 489.208), δ_H 0.824 (3 H, s, 27-H), 1.249 (3 H, s, Me), 1.362 (3 H, s, Me), 1.378 (3 H, s, Me), 2.006 (3 H, s, 23-H), 2.475 (1 H, d, *J* 18.9 Hz, 22-H_b), 2.752 (1 H, d, *J* 18.9 Hz, 22-H_a), 2.790 (1 H, s, 12-H_b), 2.805 (1 H, s, 18-H_b), 3.472 (3 H, s, 28-H), 4.072 (3 H, s, 29-H), 5.064 (1 H, d, *J* 15.2 Hz, 1-H_b), and 5.098 (1 H, d, *J* 15.2 Hz, 1-H_a); δ_C * 10.36 (C-23), 10.55 (C-27), 17.60 (C-22), 25.34, 27.14, 28.87, 38.58 T [*J* (CD), 17.3 Hz, C-21], 48.58 T [*J* (CD) 19.0 Hz, C-18], 49.55 (C-28), 50.72 T [*J* (CD) 19.1 Hz, C-12], 60.97 (C-20), 62.17 (C-29), 68.19 (C-1), 81.71 (C-11), 83.19 (C-15), 91.17 (C-14), 108.28, 114.08, 114.74, 117.91 (C-17), 145.96, 155.46, 156.79, 168.95 (C-3), 202.19 (C-19), and 203.62 (C-13).

Deuteriation of the Monoketone (**19**).—The monoketone (**19**) (25 mg), was deuteriated (see above), to give the *dideuterioketone* (**22**) (21 mg, 84%), as a white solid, with t.l.c. behaviour identical with that of the starting material (Found: M^+, 474.221. $C_{26}H_{30}{}^2H_2O_8$ requires *M*, 474.222), δ_H 0.645 (3 H, s, 27-H), 1.346 (3 H, s, Me), 1.354 (3 H, s, Me), 1.524 (3 H, s, Me), 1.759—1.799 (1 H, m), 1.836—1.900 (1 H, m), 1.972—2.048 (2 H, m), 1.999 (3 H, s, 23-H), 2.635 (1 H, s, 12-H_b), 2.817 (1 H, d, *J* 18.9 Hz, 22-H_b), 2.871 (1 H, d, *J* 18.9 Hz, 22-H_a), 3.424 (3 H, s, 28-H), 4.098 (3 H, s, 29-H), and 5.076 (2 H, s, 1-H); δ_C* 10.52 (C-23), 16.25 (C-27), 18.24 (C-22), 24.55, 27.08, 30.09, 30.26, 30.35, 36.31 T [*J* (CD) 20.5 Hz, C-21], 47.02 (C-20), 49.04 (C-28), 51.06 T [*J* (CD) 19.3 Hz, C-12], 62.08 (C-29), 68.17 (C-1), 82.15 (C-11), 82.66 (C-15), 93.47 (C-14), 107.81, 114.67, 114.68, 118.55 (C-17), 145.73, 155.29, 157.33, 169.15 (C-3), and 204.07 (C-13).

* Obtained from the broad-band proton-decoupled ^{13}C n.m.r. spectrum. The multiplicities of certain peaks indicate (C,D) coupling.

References

1 For a preliminary account of part of the work see R. M. Horak, P. S. Steyn, P. H. van Rooyen, R. Vleggaar, and C. J. Rabie, *J. Chem. Soc., Chem. Commun.*, 1981, 1265.

2 P. S. Steyn, *Tetrahedron*, 1973, **29**, 107; P. S. Steyn and R. Vleggaar, *Phytochemistry*, 1976, **15**, 355.

3 R. Vleggaar, P. S. Steyn, and D. W. Nagel, *J. Chem. Soc., Perkin Trans. 1*, 1974, 45; P. S. Steyn and R. Vleggaar, *J. Chem. Soc., Perkin Trans. 1*, 1976, 204.

4 P. S. Steyn and R. Vleggaar, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2250; *J. S. Afr. Chem. Inst.*, 1975, **28**, 375.

5 K. K. Chexal, J. P. Springer, J. Clardy, R. J. Cole, J. W. Kirksey, J. W. Dorner, H. G. Cutter, and B. J. Strawter, *J. Am. Chem. Soc.*, 1976, **98**, 6748.

6 D. F. G. Pusey and J. C. Roberts, *J. Chem. Soc.*, 1963, 3542; J. S. E. Holker, S. A. Kagal, L. J. Mulheirn, and P. M. White, *Chem. Commun.*, 1966, 911; P. Roffey and M. V. Sargent, *Chem. Commun.*, 1966, 913.

7 T. Hamasaki, Y. Hatsuda, N. Terashima, and M. Renbutsu, *Agric. Biol. Chem. (Japan)*, 1967, **31**, 11.

8 R. M. Horak, P. S. Steyn, and R. Vleggaar, *S. Afr. J. Chem.*, 1982, **35**, 60.

9 J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 1951, 877; L. A. Duncanson, J. F. Grove, and J. Zeally, *J. Chem. Soc.*, 1953, 1331.

10 W. D. Chandler, W. McFarlane Smith, and R. Y. Moir, *Can. J. Chem.*, 1964, **42**, 2549.
11 M. Karplus, *J. Am. Chem. Soc.*, 1963, **85**, 2870.
12 K. G. R. Pachler, *J. Magn. Reson.*, 1972, **7**, 442; K. G. R. Pachler, P. L. Wessels, J. Dekker, J. J. Dekker, and T. G. Dekker, *Tetrahedron Lett.*, 1976, 3059.
13 J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972.
14 K. G. R. Pachler and P. L. Wessels, *J. Magn. Reson.*, 1973, **12**, 337; 1977, **28**, 53; T. G. Dekker, K. G. R. Pachler, and P. L. Wessels, *Org. Magn. Reson.*, 1976, **8**, 530.
15 A. A. Chalmers, K. G. R. Pachler, and P. L. Wessels, *J. Magn. Reson.*, 1974, **15**, 415.
16 J. L. Marshall, 'Carbon-carbon and carbon-proton NMR couplings,' Verlag Chemie International, Florida, 1983; V. A. Chertkov and N. M. Sergeyev, *J. Am. Chem. Soc.*, 1977, **99**, 6750.
17 R. Hänni and Ch. Tamm, *J. Chem. Soc., Chem. Commun.*, 1975, 563.
18 A. E. de Jesus, R. M. Horak, P. S. Steyn, and R. Vleggaar, *J. Chem. Soc., Chem. Commun.*, 1983, 716.
19 N. J. McCorkindale, R. L. Baxter, and W. B. Turner, *Tetrahedron*, 1981, **37**, 2131.
20 T. J. Simpson and D. J. Stenzel, *J. Chem. Soc., Chem. Commun.*, 1981, 856.
21 T. J. Simpson and D. J. Stenzel, *J. Chem. Soc., Chem. Commun.*, 1982, 890.
22 T. J. Simpson, A. E. de Jesus, P. S. Steyn, and R. Vleggaar, *J. Chem. Soc., Chem. Commun.*, 1982, 632.
23 C. Abell and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1981, 856.
24 J. B. Stothers, C. T. Tan, A. Nickon, F. Huang, R. Sridhar, and R. Weglein, *J. Am. Chem. Soc.*, 1972, **94**, 8581.
25 E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, 1956, **78**, 6269.
26 J. W. ApSimon, P. V. Demarco, D. W. Mathieson, W. G. Craig, A. Karim, L. Saunders, and W. B. Whalley, *Tetrahedron*, 1970, **26**, 119.

Received 7th June 1984; *Paper* 4/944