Metabolites of *Aspergillus ustus*. Part 3. Structure Elucidation of Austalides G—L[1]

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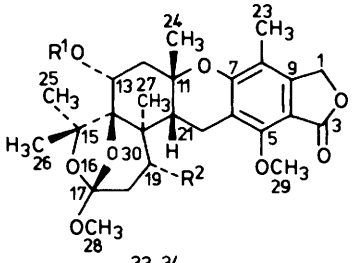
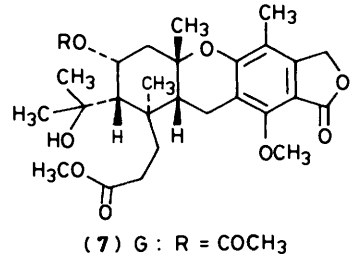
The structure elucidation of austalides G—L, based on a study of their ^1H and ^{13}C n.m.r. spectra and chemical derivatization, is described.

In an accompanying paper[2] the isolation of 12 meroterpenoid metabolites, austalides A—L, from cultures of *Aspergillus ustus* (Bainier) Thom. and Church (strain MRC 1163) and the structure elucidation of six of these metabolites, austalides A—F [(**1**)—(**6**)] are described. We now report the structure elucidation of the austalides G—L [(**7**)—(**12**)] based on a detailed study of their high-field ^1H and ^{13}C n.m.r. spectra, chemical derivatizations, and comparison with the austalides A—F.

Austalides G (**7**),† $C_{28}H_{38}O_9$, and H (**8**), $C_{26}H_{36}O_8$, are the minor metabolites in the austalide series and probably represent the products of a branch-point in the biosynthetic pathway leading to the highly oxygenated austalides A—F [(**1**)—(**6**)].[3]



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

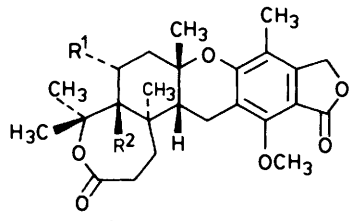


(**7**) G: $R = COCH_3$
(**8**) H: $R = H$

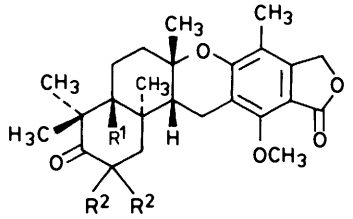
The u.v. maxima ($\lambda_{max.}$ 221 and 267 nm) of austalides G and H compare well with the corresponding data of austalides A—F. The i.r. absorption band of the C-17 carbonyl groups in the metabolites is masked by that of the phthalide carbonyl group ($\lambda_{max.}$ 1 740 cm^{-1}). The electron impact mass spectra of these two methyl esters exhibited intense $M^+ - 18$ peaks which arise through the facile loss of the elements of water from the molecular ion. This loss is consistent with the presence of the tertiary hydroxy group at C-15.

The location of the *O*-acetyl group at C-13 in austalide G (**7**) followed from the chemical shift (δ_H 5.391) of the 13-H proton. This proton is part of a four-proton spin system, the first-order analysis of which was confirmed by selective irradiation of the C-13 and C-14 protons in a series of proton–proton decoupling experiments (see Figure 1). The broad singlet at δ_H 1.593 which disappeared on addition of deuterium oxide to the sample, is assigned to the proton of the C-15 hydroxy group.

The ^{13}C n.m.r. data of austalide G are in agreement with the proposed structure. The low-field chemical shift of C-14 [δ_C 51.67, ^1J(CH) 126.2 Hz] is probably due to the inductive effect of the two oxygen atoms which are two bonds removed. The



(**9**) I: $R^1 = OCOCH_3$, $R^2 = H$
(**10**) J: $R^1 = H$, $R^2 = OH$



(**11**) K: $R^1 = R^2 = H$
(**12**) L: $R^1 = OH$, $R^2 = H$
(**18**) $R^1 = H$, $R^2 = D$
(**19**) $R^1 = OD$, $R^2 = D$

presence of an aliphatic methyl ester is evident from the singlet at δ_C 174.47 (C-17) and the quartet at δ_C 51.67 [^1J(CH) 146.6 Hz, C-28] in the single frequency n.O.e. ^{13}C spectrum.[4,5]

† The numbering of austalides G, H, K, and L is in accord with the system used for austalides A—F, and 16 is, therefore, omitted.

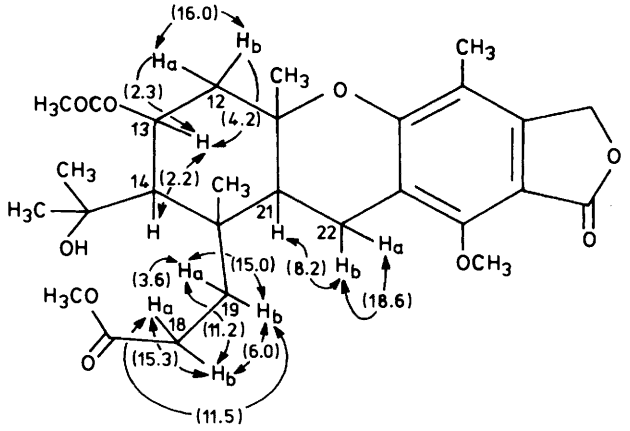


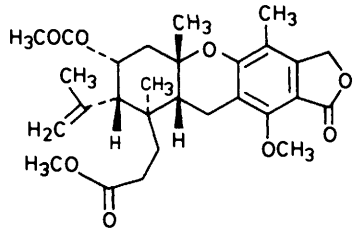
Figure 1. The (^{1}H,^{1}H) connectivity pattern of austalide G (**7**). Values in Hz

Alkaline hydrolysis of austalide G (0.1M-potassium hydroxide in methanol) yielded a single product which was identical with austalide H (**8**). Significantly, the ^{1}H n.m.r. signal of the C-13 methine proton (δ_H 4.629) of austalide H appears as an unresolved multiplet, 0.762 p.p.m. upfield in comparison to the corresponding resonance in the ^{1}H spectrum of austalide G. This signal changed to a well-resolved multiplet (J 3.7, 3.1, and 2.5 Hz) upon addition of deuterium oxide to the sample. The location of the secondary hydroxy group at C-13 was demonstrated, using the deuteriated sample, by selective irradiation of the 14-H resonance (δ_H 1.322, J 2.5 Hz) in a homonuclear decoupling experiment, which changed the 13-H signal to a double doublet (J 3.7 and 3.1 Hz).

Austalide I (**9**), m.p. 236—238 °C analysed for $C_{27}H_{34}O_8$ and had M^+ 486. The presence of the phthalide chromophore was evident from the absorption maxima at $\lambda_{max.}$ 221 and 266 nm in the u.v. spectrum. The i.r. spectrum of the metabolite had $\nu_{max.}$ 1 720—1 750 cm^{-1}, assigned to the C-3, C-33, and C-17 carbonyl groups.

The presence of the *O*-acetyl moiety at C-13 in austalide I was inferred from the chemical shift of the 13-H proton resonance (δ_H 5.407, m). This resonance changed to a double doublet (J 4.2, 1.9 Hz) on selective irradiation of the doublet signal (δ_H 1.862, J 3.9 Hz) assigned to 14-H.

The single frequency n.O.e. ^{13}C n.m.r. spectrum of austalide I showed the presence of three doublets at δ_C 45.94 [^1J(CH) 125.4 Hz], 55.35 [^1J(CH) 120.6 Hz], and 69.92 [^1J(CH) 151.0 Hz]. The chemical shift and coupling constant of the last resonance are diagnostic of the oxygen-bearing carbon atom, C-13. The assignment of the doublet signals at δ_C 45.94 (C-21) and 55.35 (C-14) is based on chemical-shift considerations. The chemical shift of the resonance at δ_C 174.40, ascribed to C-17, compares well with the chemical shift of the carbonyl carbon atom in seven-membered lactones.[4] The facile ring-opening of seven-membered lactones on treatment with acid is well documented and can be taken as proof of the presence of such a functionality.[6,7] For this reason, a solution of austalide I (**9**) in a mixture of dichloromethane and methanol was treated with anhydrous hydrogen chloride generated *in situ* by the addition of thionyl chloride to the reaction mixture.[8] After 2 h at room temperature the ester (**13**), M^+ 500, was obtained in 73% yield. Significant features of the ^{1}H n.m.r. spectrum of (**13**) in comparison with that of austalide I are the unresolved multiplets at δ_H 4.893 and 4.939 ascribed to the protons of the exocyclic methylene group. A three-proton singlet at δ_H 3.683 is indicative of the protons of a methyl ester. It is evident that under these experimental conditions opening of the lactone ring in austalide I leads firstly to the formation of austalide G (**7**).

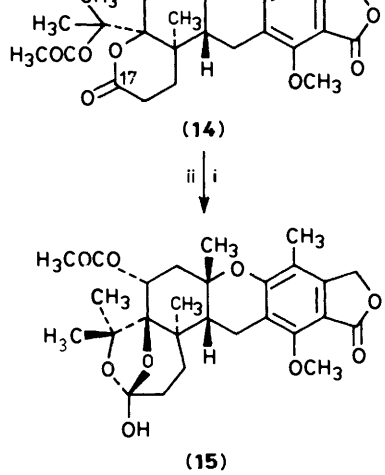


(**13**)

The facile loss of water from the hydroxy-isopropyl group in (**7**) then generates the exocyclic methylene moiety.

Austalide J (**10**), $C_{25}H_{32}O_7$, has the characteristic u.v. and i.r. data of austalides A—I, and M^+ 444. Although the molecular ions of austalide I (**9**) and J (**10**) differ by 42 mass units, austalide J is not simply the deacetyl derivative of austalide I. This was apparent from a comparison of the respective ^{1}H and ^{13}C n.m.r. data. The resonances at δ_H 1.862 and 5.407, assigned respectively to the C-14 and C-13 protons in austalide I were both absent in the ^{1}H spectrum of austalide J. The ^{13}C n.m.r. spectrum of austalide J showed three resonances (δ_C 75.85, 79.58, and 91.33) due to oxygen-bearing, quaternary, sp^3 carbon atoms but none of which could be assigned to an oxygen-bearing methine carbon atom. Austalide J is, therefore, oxygenated at C-14, and as a result it can contain either a six- or a seven-membered lactone ring. This ambiguity was resolved by the chemical shift (δ_C 173.38) of the lactone carbonyl carbon atom. The corresponding carbon atom in six-membered lactone rings resonates at *ca.* δ_C 167.[4]

In the preceding paper[1] it is shown that treatment of the lactone (**14**) with a methanolic solution of potassium hydroxide followed by acidic work-up leads to the formation of the hemi-ortho ester (**15**) in high yield (see Figure 2). Austalide J could



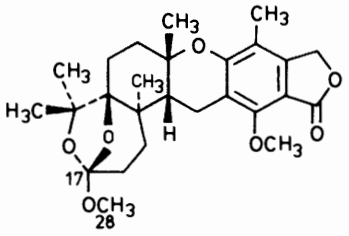
(**14**)

ii | i

(**15**)

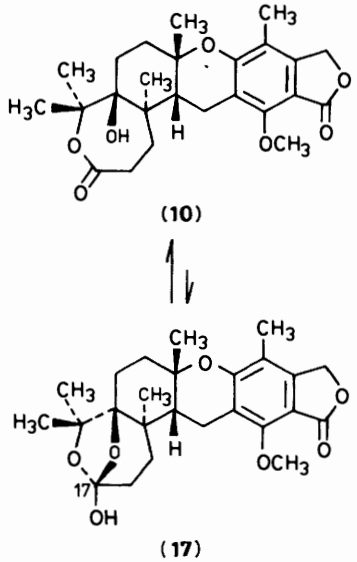
Figure 2. Transformation of the δ-lactone (**14**) into the hemi-ortho ester (**15**). (i) KOH–MeOH, (ii) 0.1M HCl

conceivably exist in solution in equilibrium with its hemi-ortho ester form (**17**) which could be trapped by methylation. Although the broad-band proton-decoupled ^{13}C n.m.r. spectrum of austalide J in both chloroform and methanol is consistent in each case only with the lactone form (**10**), the ortho ester (**16**) was obtained in 90% yield when austalide J was treated with hydrogen chloride in anhydrous methanol. The same product was also formed by methylation of austalide J



(16)

with methyl iodide and potassium carbonate in dry acetone. The efficient conversion of austalide J into its ortho ester form proceeds in each case to completion as methylation of (**17**) shifts the equilibrium between (**10**) and (**17**).



(10)

(17)

Figure 3. Proposed equilibrium between austalide J (**10**) and its hemi-ortho ester (**17**)

The ^{1}H n.m.r. spectrum of (**16**) displayed a three-proton singlet at δ_H 3.411, characteristic of the ortho ester methyl protons (28-H) in the austalides A—F [(**1**)—(**6**)]. The corresponding methyl carbon atom gives rise to a signal at δ_C 48.74 in the ^{13}C n.m.r. spectrum.

The facile transformation of austalide J into the ortho ester (**16**) indicates that the compound has the same relative configuration as austalide D (14*S*) and provides chemical proof of the structure. The change in descriptor for this chiral centre compared with the corresponding chiral centre in austalide D is merely the result of the sequence rules of the Cahn-Ingold-Prelog system.

Austalide K (**11**), $C_{25}H_{32}O_5$, and austalide L (**12**), $C_{25}H_{32}O_6$, have u.v. data similar to those of austalides A—J. The i.r. spectra of each of these two metabolites showed an absorption band at $\nu_{max.}$ 1 700 cm^{-1}, characteristic of a carbonyl group (C-17) in a six-membered ring.[9]

A number of signals in the ^{1}H n.m.r. spectrum of austalide K (**11**) exhibited extensive fine structure as a result of multiple geminal and vicinal proton–proton coupling (Figure 4). The resonances of the C-12, C-13, and C-14 protons appear as deceptively simple multiplets in the ^{1}H n.m.r. spectrum since one vicinal and both geminal proton–proton coupling constants have the same magnitude. Extensive ^{1}H-{^{1}H} homonuclear decoupling experiments were, therefore, necessary to analyse the various multiplets and assign them to specific protons. The resultant proton–proton connectivity pattern is illustrated in

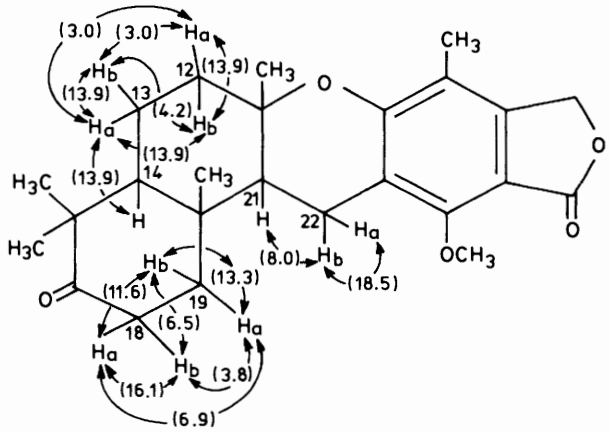


Figure 4. The (^{1}H,^{1}H) connectivity pattern of austalide K (**11**). Values in Hz

Figure 4. The 13-H$_a$ proton is assigned to the eight-line multiplet at δ_H 1.785 (*J* 13.9, 13.9, 13.9, and 3.0 Hz) since it is the only proton with an antiperiplanar orientation with two different neighbouring protons (12-H$_b$ and 14-H). The magnitude of the geminal coupling constant of the C-18 protons can be explained by the presence of a carbonyl group at C-17 (see Figure 4).[10]

A singlet resonance at δ_C 216.23 in the ^{13}C n.m.r. spectrum of austalide K was assigned to the carbonyl carbon atom, C-17. The corresponding carbon aton in 4,4-dimethyl-3-ketosteroids resonates at *ca.* δ_C 216.[11] The chemical-shift values of the C-14 [δ_C 54.07 D, ^1J(CH) 120.9 Hz] and C-15 (δ_C 47.09 S) resonances confirmed the lack of oxygenation at these centres.

The structural assignment of austalide K (**11**) is corroborated by the results obtained from an n.m.r. study of its deuteriated derivative (**18**), obtained by refluxing the compound in a solution of sodium methoxide in [*O*-^{2}H]methanol.[12] As a result of the base-catalysed ring-opening of the phthalide moiety in the compound, the reaction mixture was acidified during work-up with deuteriated acid obtained from a 1:1 mixture of deuterium oxide and acetyl chloride. A comparison of the ^{1}H n.m.r. spectrum of (**18**) with that of austalide K shows that the signals assigned to the C-18 protons are absent. In addition, the C-19 protons give rise to an AX spin system (*J* 13.3 Hz).

The broad-band proton-decoupled ^{13}C n.m.r. spectrum of the deuteriated derivative (**18**) showed 24 singlet signals plus an ill-resolved multiplet (δ_C 33.50) of very low relative intensity. This multiplet, assigned to C-18, is centred upfield ($\Delta\delta$ −0.41 p.p.m.) from the resonance position of C-18 in the spectrum of austalide K (**11**).[13] The relatively low intensity of the C-18 signal in the spectrum of (**18**) is due to the loss of n.O.e. and the fact that it is split into five lines as a result of spin coupling with the two deuterium atoms.[14] Furthermore, the C-19 signal exhibits an upfield shift ($\Delta\delta$ −0.08 p.p.m.) whereas the C-17 resonance is shifted downfield ($\Delta\delta$ 0.13 p.p.m.).[12,13] These isotope shifts support the structural assignment of austalide K(**11**).

The structural assignment of austalide L(**12**) is based on the same approach as described for austalide K. First-order analysis of the multiplets in the ^{1}H n.m.r. spectrum of austalide L yielded the chemical shifts and coupling constants. The values of the coupling constants, as corroborated by ^{1}H-{^{1}H} decoupling experiments, indicated the geminal and vicinal proton-proton coupling patterns in the molecule, as outlined in Figure 5.

The deuteriated derivative (**19**) of austalide L was obtained as described above for austalide K. The presence of three deuterium atoms in the molecule is evident from the mass

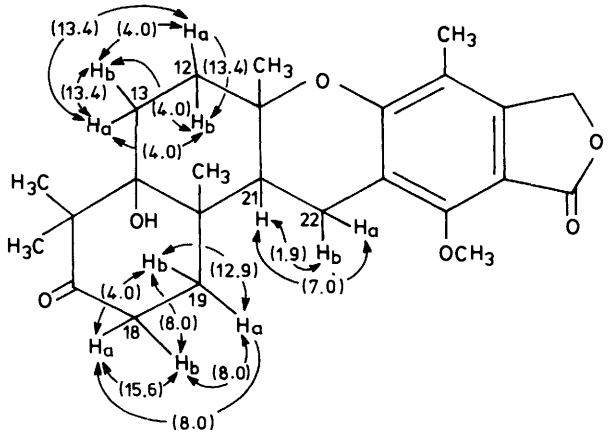


Figure 5. The (^{1}H,^{1}H) connectivity pattern of austalide L (**12**). Values in Hz

spectral data. The ^{1}H n.m.r. spectrum lacks the signals assigned to the C-14 hydroxy group and the C-18 methylene protons, and the signal assigned to the C-19 protons of (**19**) appears as a pair of doublets (*J* 12.9 Hz).

A comparison of the ^{13}C n.m.r. data of the trideuterioketone (**19**) and of austalide L (**12**) indicated an upfield shift (Δδ −0.10 p.p.m.) for the C-19 resonance whereas the C-14 signal is shifted downfield (Δδ 0.11 p.p.m.). Deuteriation at C-18 in (**19**) causes an upfield shift (Δδ −0.15 p.p.m.) instead of a downfield shift[15] [see austalide K (**11**)] in the resonance position of the C-17 carbonyl carbon atom due to the presence of the C-14 hydroxy group.

The austalides, *e.g.* austalide D (**4**), are biosynthetically derived from 6-farnesyl-5,7-dihydroxy-4-methylphthalide, a known intermediate in the biosynthesis of mycophenolic acid,[16] by stereospecific ring closure to give austalide K (**11**).[3] Hydroxylation at C-14 of austalide K (**11**) proceeds with retention of configuration to give austalide L (**12**). An enzymatic Baeyer-Villiger oxidation (retention of configuration)[17] produces the seven-membered hydroxy-lactone ring of austalide J (**10**) with the correct stereochemical orientation of the substituents for *in vivo* cyclisation to an ortho ester. This cyclisation was demonstrated *in vitro* for austalide J (**10**) (see above).

The detailed biosynthetic studies on the austalides will be described in a subsequent publication.

Experimental

For general directions see reference 1.

Alkaline Hydrolysis of Austalide G (**7**).—Austalide G (10 mg) was hydrolysed according to the procedure described in reference 1 to give the diol (**8**) (8 mg, 87%), identical with an authentic sample of austalide H (**8**).

Acid-catalysed Transformation of Austalide I (**9**).—Thionyl chloride (1 ml) was added to a cooled solution of austalide I (8 mg) in dichloromethane–methanol (1:1 v/v; 10 ml) and the solution was stirred for 2 h at room temperature. The reaction mixture was diluted with ether (50 ml), washed with water (2 × 50 ml), saturated aqueous sodium hydrogen carbonate (2 × 50 ml), and water (3 × 50 ml), and dried ($MgSO_4$). Evaporation of the solvent gave the *ester* (**13**) (6 mg, 73%) as a white amorphous solid, $[\alpha]_D$ −69.7° (*c* 1.00), $\lambda_{max.}$ 222 and 267 nm (ε 26 100 and 11 700, respectively), $\nu_{max.}$ 1 740 cm^{-1} (Found: M^+, 500.241. $C_{28}H_{36}O_8$ requires *M*, 500.241), δ_H 0.880 (3 H, s, 27-H), 1.190 (3 H, s, Me), 1.627 (1 H, d, *J* 8.0 Hz, 21-H), 1.666—1.739 (2 H, m), 1.757—1.819 (1 H, m), 1.811 (3 H, s, Me), 1.986 (3 H, s, Me), 2.023 (3 H, s, Me), 2.160 (1 H, d, *J* 2.5 Hz, 14-H), 2.264—2.298 (2 H, m), 2.583 (1 H, dd, *J* 15.8 and 2.2 Hz), 2.776 (1 H, dd, *J* 18.4 and 8.0 Hz, 22-H_b), 2.934 (1 H, d, *J* 18.4 Hz, 22-H_a), 3.683 (3 H, s, 28-H), 4.117 (3 H, s, 29-H), 4.893 (1 H, m br, 25-H_a), 4.939 (1 H, m br, 25-H_b), 5.080—5.091 (1 H, m, 13-H), and 5.096 (2 H, s, 1-H).

Acid-catalysed Dehydration of Austalide G (**7**).—Thionyl chloride (1 ml) was added to a cooled solution of austalide G (5 mg) in dichloromethane–methanol (1:1 v/v; 5 ml) and the solution was stirred for 2 h at room temperature. Work-up as before yielded a compound (4 mg, 83%) identical with an authentic sample of the ester (**13**).

Acid-catalysed Transformation of Austalide J (**10**).—Thionyl chloride (1 ml) was added to a cooled solution of austalide J (40 mg) in dichloromethane–methanol (1:1 v/v; 10 ml) and the solution was stirred for 2 h at room temperature. Work-up as before and recrystallisation from acetone gave the *ortho ester* (**16**) (37 mg, 90%) as white needles, m.p. 239—241 °C, $[\alpha]_D$ −51.2° (*c* 1.00), $\lambda_{max.}$ 222 and 266 nm (ε 27 800 and 12 100, respectively), $\nu_{max.}$ 1 745 cm^{-1} (Found: M^+, 458.229. $C_{26}H_{34}O_7$ requires *M*, 458.231), δ_H 0.767 (3 H, s, 27-H), 1.202 (3 H, s, Me), 1.332 (3 H, s, Me), 1.445 (3 H, s, Me), 1.669 (1 H, ddd, *J* 13.7, 4.5, and 2.7 Hz), 1.696—1.744 (2 H, m), 1.818—1.923 (2 H, m), 1.945 (1 H, ddd, *J* 13.7, 13.7, and 4.5 Hz), 2.008 (3 H, s, 23-H), 2.039—2.136 (2 H, m), 2.365 (1 H, dd, *J* 7.3 and 1.6 Hz, 21-H), 2.810 (1 H, dd, *J* 18.8 and 7.3 Hz, 22-H_b), 2.844 (1 H, dd, *J* 18.8 and 1.6 Hz, 22-H_a), 3.411 (3 H, s, 28-H), 4.095 (3 H, s, 29-H), and 5.093 (2 H, s, 1-H); δ_C 10.59 Q (C-23), 17.98 Q (C-27), 18.39 T (C-22), 22.01 Q, 24.84 Q, 27.14 Q, 29.73 T, 29.91 T, 30.78 T, 34.71 T, 36.48 D (C-21), 40.78 S (C-20), 48.74 Q (C-28), 61.93 Q (C-29), 68.20 T (C-1), 76.48 S (C-11), 83.94 S, 88.34 S, 107.33 S, 114.31 S, 115.97 S, 118.67 S (C-17), 145.44 S, 155.38 S, 158.52 S, and 169.37 S (C-3).

Treatment of Austalide J (**10**) *with Methyl Iodide*.—Austalide J (10 mg) in dry acetone (100 ml) was refluxed with methyl iodide (1 ml) in the presence of potassium carbonate. After 65 h (t.l.c. control) the reaction mixture was filtered and evaporated under reduced pressure to give a white amorphous solid. Crystallization of this material from acetone yielded the ortho ester (8 mg, 78%) as white needles, identical with an authentic sample of (**16**).

Deuteriation of Austalide K (**11**).—Austalide K (50 mg) was deuteriated according to the procedure described in reference 2 to give the deuterioketone (**18**) (47 mg, 94%) as a white glass, with t.l.c. behaviour identical with that of the starting material (Found: M^+, 414.236. $C_{25}H_{30}{}^2H_2O_5$ requires *M*, 414.238), δ_H 0.698 (3 H, s, 27-H), 0.999 (3 H, s, Me), 1.090 (3 H, s, Me), 1.163 (3 H, s, Me), 1.478 (1 H, d, *J* 13.3 Hz, 19-H_b), 1.482 (1 H, dm, *J* 13.9 Hz, 14-H), 1.483 (1 H, d, *J* 8.0 Hz, 21-H), 1.512 (1 H, dm, *J* 13.9 Hz, 13-H_b), 1.617 (1 H, ddd, *J* 13.9, 13.9, and 4.2 Hz, 12-H_b), 1.789 (1 H, dddd, *J* 13.9, 13.9, 13.9, and 3.5 Hz, 13-H_a), 2.016 (3 H, s, 23-H), 2.075 (1 H, d, *J* 13.3 Hz, 19-H_a), 2.263 (1 H, ddd, *J* 13.9, 3.0, and 3.0 Hz, 12-H_a), 2.790 (1 H, dd, *J* 18.5 and 8.0 Hz, 22-H_b), 2.913 (1 H, d, *J* 18.5 Hz, 22-H_a), 4.081 (3 H, s, 29-H), and 5.087 (2 H, s, 1-H); δ_C* 10.51 (C-23), 14.06 (C-27), 18.15 (C-22), 21.51, 26.61, 26.96, 29.54, 33.50 M (C-18), 37.49 (C-20), 38.24, 39.68 (C-19), 46.95 (C-21), 47.09 (C-15), 54.07 (C-14), 61.75 (C-29), 68.07 (C-1), 76.20 (C-11), 107.18, 114.29, 115.14, 145.38, 155.23, 158.45, 169.17 (C-3), and 216.36 (C-17).

Deuteriation of Austalide L (**12**).—Austalide L (30 mg) was deuteriated (as above) to give the *deuterioketone* (**19**) (26 mg, 87%) as white plates, m.p. 206—207 °C (from benzene–

n-hexane), with t.l.c. behaviour identical with that of the starting material (Found: M^+, 431.238. $C_{25}H_{29}{}^2H_3O_6$ requires M, 431.239), δ_H 0.781 (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), 1.747 (1 H, d, J 12.9 Hz, 19-H_b), 1.970—2.028 (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), 2.156 (1 H, ddd, J 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), 2.736—2.829 (2 H, m, 22-H), 4.064 (3 H, s, 29-H), and 5.077 (2 H, s, 1-H); δ_C* 10.61 (C-23), 18.04 (C-22), 18.26 (C-27), 21.62, 23.57, 24.19, 26.81, 33.06 (C-19), 33.39, *ca.* 33.3 (C-18),† 40.72 (C-21), 41.23 (C-20), 56.62 (C-15), 61.85 (C-29), 68.16 (C-1), 76.12 (C-11), 79.64 (C-14), 107.23, 114.40, 115.84, 145.41, 155.29, 158.59, 169.35 (C-3), and 216.25 (C-17).

* Obtained from the broad band proton-decoupled ^{13}C n.m.r. spectrum. The multiplicities of certain peaks indicate (C,D) coupling.
† Appears as a weak, partly obscured multiplet.

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Received 7th June 1984; *Paper* 4/946