Studies on Fungal Metabolites. Part 3.[1] ^{13}C N.m.r. Spectral and Structural Studies on Austin and New Related Meroterpenoids from *Aspergillus ustus*, *Aspergillus variecolor*, and *Penicillium diversum*

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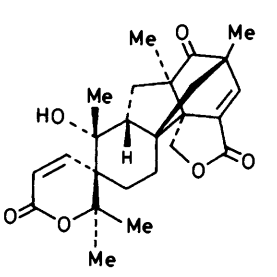
The ^{13}C n.m.r. spectrum of austin has been fully assigned by *inter alia* analysis of long range ^{1}H–^{13}C couplings in the fully ^{1}H-coupled ^{13}C n.m.r. spectrum. Further related metabolites, dehydroaustin, austinol, and isoaustin, have been isolated from *Aspergillus ustus*, *Aspergillus variecolor*, and *Penicillium diversum*. Their structures have been assigned by detailed analysis of their ^{1}H and ^{13}C n.m.r. spectra.

THE andibenins, *e.g.* (1) and andilesins are a series of related compounds isolated from *Aspergillus variecolor*.[2] They have been shown by ^{13}C- and ^{2}H-labelling studies to be of mixed polyketide–terpenoid origins [3] and the suggestion was made that the mycotoxins austin (2) and terretonin (3), metabolites of *Aspergillus ustus* [4] and *Aspergillus terrus* [5] respectively, for which sesterterpenoid origins have been proposed, might be biosynthetically related to them. In this paper we report detailed ^{13}C n.m.r. studies on austin and the application of these studies to assigning the structures of further closely related metabolites isolated from *A. variecolor*, *A. ustus*, and *Penicillium diversum*.

The chemical shifts observed in the proton noise decoupled (p.n.d.) ^{13}C n.m.r. spectrum of austin, and the multiplicities observed in the single frequency off-resonance decoupled (s.f.o.r.d.) spectrum are summarised in Table 1. Chemical shift considerations and the observed multiplicities allowed the assignment of the resonances at 146.5, 120.2, and 118.0 p.p.m. to C-1, -2, and -1′ respectively. The triplet at 27.0 p.p.m. in the s.f.o.r.d. spectrum could be assigned to C-6 as the remaining methylene carbon C-7 gives a doublet of doublets at 26.5 p.p.m. due to the large difference in the chemical shifts of the 7-methylene protons (Table 2). However, due to the complexity of the structure and the lack of suitable models, the task of assigning the remaining resonances appeared formidable, with the main problems being differentiating among the four carbonyl resonances, the five resonances due to oxygen-bearing sp^3-hybridised carbons, and the seven resonances due to *C*-methyls. The problem was solved by analyses of long range ^{1}H–^{13}C couplings in the fully ^{1}H-coupled ^{13}C n.m.r. spectrum using low power specific ^{1}H decouplings and ^{2}H exchange. While these methods have been applied extensively to aromatic compounds,[6] their application to complex alicyclic compounds is novel and is made possible by use of very high field spectrometers where, in addition to the higher sensitivity and dispersion of the ^{13}C n.m.r. spectrum, the extra dispersion of the ^{1}H spectrum allows specific irradiation of the protons to be carried out more easily.

Figure 1 shows the appearance of the fully ^{1}H-coupled ^{13}C n.m.r. spectrum in the carbonyl and oxygen-bearing aliphatic regions, and the effect on the spectrum of a number of specific decoupling and exchange experiments. Each of the four carbonyl resonances gives a distinctive



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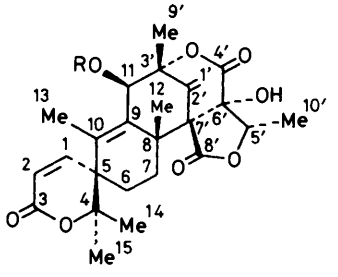
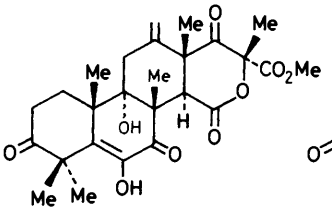


(2) R = Ac
(5) R = H



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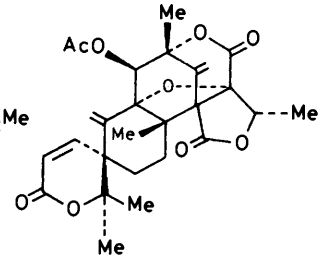

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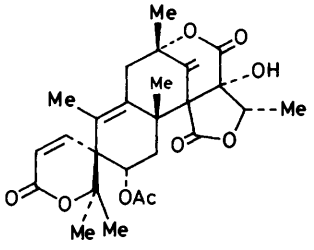


(6)

signal. C-3 Appears as a doublet of doublets, the observed 11 and 5 Hz splittings being removed in turn by irradiation of 1-H and 2-H respectively. The acetate carbonyl appears as a quartet of doublets which on ir-

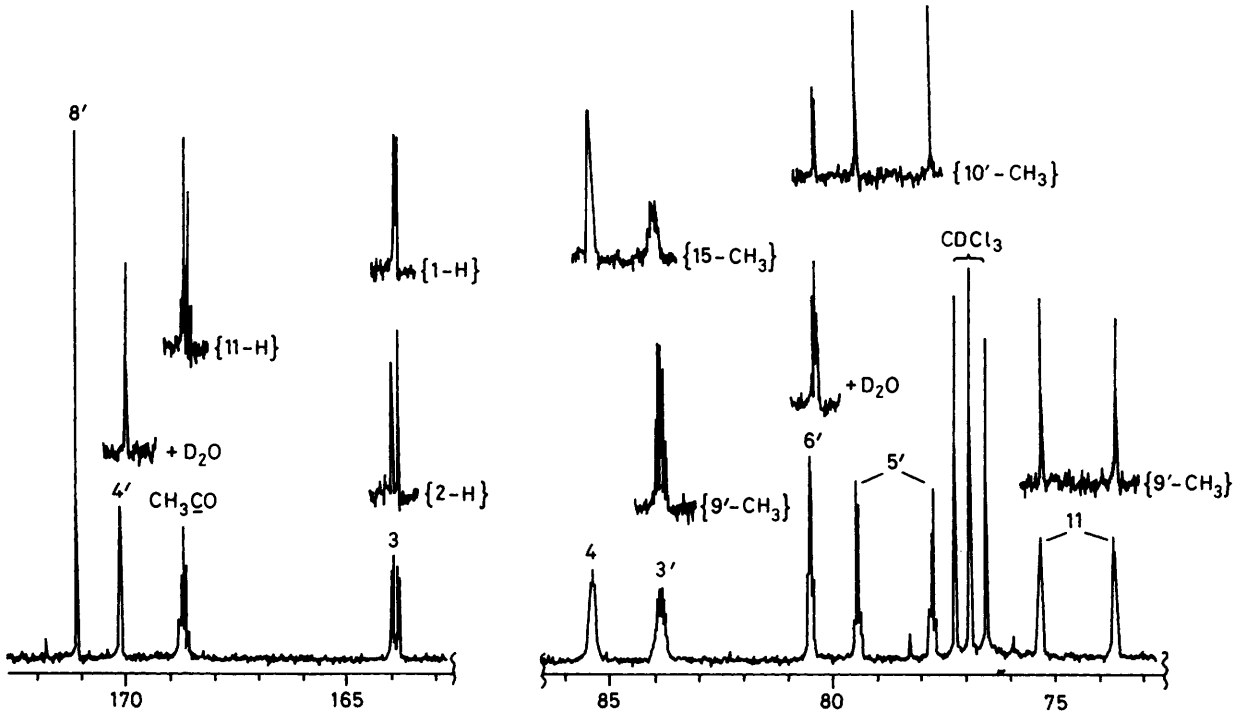


FIGURE 1 The 73—86 and 163—172 p.p.m. regions of the fully ^1H-coupled ^{13}C n.m.r. spectrum of austin, and results of D_2O exchange and selective low power decoupling experiments

TABLE 1

^{13}C N.m.r. spectra of austin and related metabolites

Carbon atom	Compound (2) δ_C [a]	(2) $^1J_{^{13}C,H}$	(2) $>^1J_{^{13}C,H}$	(4) δ_C [a]	(5) δ_C [a]	(6) δ_C [a]
1	146.5 (Ddd)	165	6,3	150.8 (D)	147.9 (S)	144.5 (D)
2	120.2 (D)	171		115.9 (D)	118.2 (S)	119.9 (D)
3	163.6 (Sdd)		11,5	163.1 (S)	163.7 (S)	163.7 (S)
4	85.5 (Sm)		b	86.0 (S)	85.6 (S)	85.6 (S)
5	46.6 (Sm)		b	50.4 (S)	45.7 (S)	50.4 (S)
6	27.0 (Tm)	129	b	27.0 (T)	26.1 (T)	70.3 (D)
7	26.5 (DDm)	129		26.5 (T)	26.1 (T)	32.1 (T)
8	42.1 (Sm)		b	44.0 (S)	41.4 (S)	42.4 (S)
9	132.6 (Sm)		b	90.5 (S)	135.6 (S)	132.5 (S)
10	143.8 (Sm)		b	139.2 (S)	138.3 (S)	138.9 (S)
11	74.7 (Dq)	153	4	74.1 (D)	73.5 (D)	42.0 (T)
12	23.5 (Qt)	125	5	16.9 (Q)	23.7 (Q)	23.6 (Q)
13	15.4 (Q)	128		125.4 (T)	13.9 (Q)	16.0 (Q)
14	22.4 (Qq)	127	4	23.7 (Q)	22.7 (Q)	23.7 (Q)
15	25.9 (Qq)	127	4	25.6 (Q)	25.8 (Q)	26.8 (Q)
1′	118.0 (T)	141		114.6 (T)	115.9 (T)	115.6 (T)
2′	137.5 (Sm)		b	137.1 (S)	137.7 (S)	134.0 (S)
3′	84.1 (Sdt)		10,5	82.5 (S)	85.2 (S)	83.5 (S)
4′	170.1 (Sd)		4	167.3 (S)	168.9 (S)	170.7 (S)
5′	78.7 (Dq)	150	5	76.3 (D)	78.6 (D)	79.0 (D)
6′	80.6 (S sex)		4	84.9 (S)	80.5 (S)	80.6 (S)
7′	62.8 (Sm)		b	64.3 (S)	62.8 (S)	62.7 (S)
8′	170.8 (S)			168.9 (S)	171.4 (S)	170.8 (S)
9′	20.2 (Q)	130		18.9 (Q)	20.7 (Q)	22.5 (Q)
10′	11.3 (Q)	129		13.3 (Q)	11.7 (Q)	11.5 (Q)
CH_3CO	168.4 (Sqd)		7,5	168.3 (S)	—	169.6 (S)
CH_3CO	20.6 (Q)	129		20.5 (Q)	—	21.4 (Q)

[a] Capital letters refer to one-bond couplings observed in s.f.o.r.d. spectra: S, singlet; D, doublet; T, triplet; Q, quartet. Lower case letters refer to long range couplings observed in fully ^1H-coupled ^{13}C n.m.r. spectra: d, doublet; triplet; m, multiplet; q, quartet; sex, sextet. [b] Couplings not resolved.

radiation of 11-H simplifies to a quartet, the remaining coupling being the two-bond coupling to the methyl protons. Irradiation of the hydroxy-proton at 4.19 p.p.m. also causes collapse of the doublet at 170.1 p.p.m. to a sharp singlet allowing its assignment to C-4′. This doublet splitting is also removed by addition of D_2O and a concomitant γ-deuterium isotope shift of −0.04 p.p.m. is observed. The remaining carbonyl resonance at 170.8 p.p.m. which appears as a sharp singlet showing no long range coupling at all, must be assigned to C-8′. Somewhat surprisingly, neither the C-4′ nor C-8′ carbonyl doublets of doublets on removal of the quartet splitting to the 9′-methyl protons. Irradiation of the 9′-methyl protons also causes the multiplet at 137.5 p.p.m. to collapse to a doublet, allowing its assignment to C-2′. The diffuse multiplet at 85.5 p.p.m. must, by elimination, be due to C-4, and this multiplet shows a strong intensity increase on irradiation of the methyl resonances at either 1.38 or 1.53 p.p.m., which are therefore assigned to the 14 and 15-methyls. Irradiation of the methyl resonance at 1.85 p.p.m. results in sharpening of the diffuse multiplets at 132.6 and 143.8 p.p.m. but the final

TABLE 2

^{1}H N.m.r. spectra of austin and related compounds

	(2) [a]	(4) [a]	(5) [b]	(6) [a]
1	6.72 (d, 9.9 Hz)	6.82 (d, 9.9 Hz)	6.73 (d, 9.8 Hz)	6.75 (d, 10.0 Hz)
2	6.11 (d, 9.9 Hz)	5.89 (d, 9.9 Hz)	5.98 (d, 9.8 Hz)	6.13 (d, 10.0 Hz)
6α	*	*	*	—
6β	*	*	*	4.89 (dd, 13.1, 3.9 Hz)
7α	3.21 (ddd, 13.2, 13.2, 3.0 Hz)	*	3.28 m	3.16 (dd, 13.2, 13.1 Hz)
7β	*	*	*	1.94 (dd, 13.2, 3.9 Hz)
11α	6.03 [c]	5.63 [c]	4.59 (d, 5.5 Hz)	3.18 (d, 16.3 Hz)
11β	—		—	2.38 (dq, 16.3, 1.6 Hz)
13-CH_2	—	5.73	—	—
1′	5.49 (d, 1.6 Hz)	5.87 (d, 1.8 Hz)	5.16 (d, 1.6 Hz)	5.32 (d, 1.5 Hz)
	5.76 (d, 1.6 Hz)	6.13 (d, 1.8 Hz)	5.61 (d, 1.6 Hz)	5.62 (d, 1.5 Hz)
5′	4.46 (q, 6.5 Hz)	5.28 (q, 6.9 Hz)	4.41 (q, 6.6 Hz)	4.37 (q, 6.3 Hz)
12-Me [d]	1.19	1.28	1.27	1.23
13-Me [d]	1.85	—	1.63	1.66 (d, 1.6 Hz)
14-Me [d]	1.53	1.40	1.45	1.43
15-Me [d]	1.38	1.46	1.39	1.29
9′-Me [d]	1.61	1.53	1.57	1.64
10′-Me	1.29 (d, 6.5 Hz)	1.61 (d, 6.7 Hz)	1.13 (d, 6.6 Hz)	1.26 (d, 6.3 Hz)
OAc [d]	2.02	1.99	—	1.97
OH [e]	4.19 [c]	—	5.77 (d, 5.5 Hz)	3.74 [e]
Others *	1.5—1.8 (3 H, m)	1.2—2.1 (4 H, m)	1.5—1.9 (3 H, m)	—

[a] $CDCl_3$ solution. [b] $(CD_3)_2CO$ solution. [c] 1 H, singlet. [d] 3 H, singlet. [e] Exchanges with D_2O.

shows any coupling to 5′-H. However, see the following discussion of dehydroaustin.

Similar decoupling experiments permit the resonances in the 74—85 p.p.m. region to be rigorously assigned. Irradiation of either the hydroxy-proton at 4.19 p.p.m. or 5′-H at 4.46 p.p.m. causes the sextet (J 4 Hz) at 80.6 p.p.m. to change to a pentet allowing its assignment to C-6′. On addition of D_2O the sextet similarly changes to a pentet with a concomitant upfield shift of 0.05 p.p.m. and on further irradiation of the 10′-methyl protons at 1.29 p.p.m., the pentet collapses to a doublet (J 4 Hz), the residual coupling being to 5′-H. Irradiation of the 10′-methyl protons also changes the doublet of quartets (J 150 and 5 Hz) centred at 78.7 p.p.m., to a simple doublet, allowing its assignment to C-5′. C-11 Appears as a doublet of poorly resolved quartets (J 153 and 4 Hz) centred at 74.7 p.p.m., and irradiation of the methyl protons at 1.61 p.p.m. removes the quartet splitting. This methyl signal must therefore be due to the 9′-methyl showing a three-bond coupling to C-11. Irradiation at 1.61 p.p.m. also causes the complex multiplet at 84.1 p.p.m. to simplify to a 5 line multiplet, as indicated in Figure 1. Further specific decoupling experiments indicate that this multiplet arises from couplings of 10 and 5 Hz to the 1′-methylene protons and a further coupling of 5 Hz to 11-H giving in essence an overlapping doublet of unambiguous assignment of the C-9 and C-10 resonances comes from the ^{13}C–^{13}C couplings of 46 and 43 Hz to C-13 and C-11 respectively, observed in the ^{13}C spectrum of austin enriched biosynthetically from sodium [1,2-$^{13}C_2$] acetate.[7]

Observation of the effect on the ^{13}C n.m.r. spectrum of irradiation of the methyl proton signals as described above results in an unambiguous assignment of the methyl region of the ^{1}H spectrum. Correlation of the ^{1}H and ^{13}C resonances by carrying out a Feeney plot experiment [8] indicated that the proton resonances at 1.19, 1.29, 1.38, 1.53, 1.61, 1.85, and 2.02 p.p.m. are coupled to the carbon resonances at 23.5, 11.3, 25.9, 22.4, 20.2, 15.4, and 20.6 p.p.m., respectively, and thus permits a rigorous assignment of the methyl signals in the ^{13}C spectrum.

The connectivity patterns revealed by detection of long range ^{1}H–^{13}C couplings as described above are summarised in Figure 2(*a*) and, as well as giving invaluable spectral assignment data in a molecule of known structure, they can be a source of invaluable structural information in molecules of uncertain structure, particularly those where, as in austin, the protons are highly insulated and the structural information available from ^{1}H–^{1}H coupling data is limited. This is illustrated below.

Two further compounds have been isolated from *A.*

ustus. These are dehydroaustin, $C_{27}H_{30}O_9$, and austinol, $C_{25}H_{30}O_8$, for which structures (4) and (5) have been established. Dehydroaustin has also been isolated along with austin from a chance mutant of the andibenin-producing culture of *A. variecolor* which no longer produced andibenin. This is a very significant observation

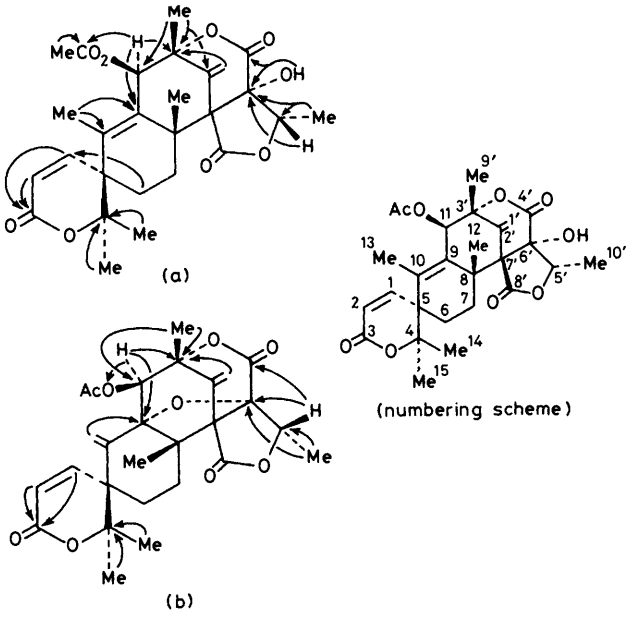


FIGURE 2 Long range ^1H–^{13}C couplings in austin and dehydroaustin selectively removed by low power decoupling experiments

in view of the proposed common biosynthetic origins of austin and andibenin B.

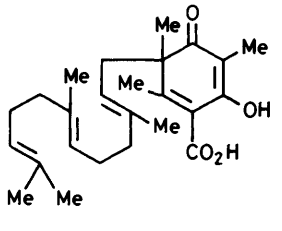
Comparison of the ^1H and ^{13}C n.m.r. spectra of austin and dehydroaustin indicated a close similarity between the two compounds. In the ^{13}C n.m.r. spectra, the signals assigned to the C-9 and C-10 olefinic and C-13 methyl carbons in austin are replaced by signals at 139.2 (S), 125.4 (T), and 90.5 (S) p.p.m. attributable to olefinic methylene and quaternary, and allylic oxygen-bearing sp^3-hybridised carbons. These differences are paralleled in the ^1H n.m.r. spectra. As both the ^1H n.m.r. and i.r. spectra indicate the absence of hydroxy-groups in dehydroaustin and the molecular formula indicates that dehydroaustin contains two less hydrogens than austin, the allylic ether structure (4) is indicated. The *X*-ray crystal structure of austin reveals that the 6′-hydroxy-oxygen is only 2.12 Å from the 7α-proton and so the proton is severely deshielded.[4] Examination of Dreiding models shows that formation of the ether linkage to C-9 in dehydroaustin requires a conformational change in the D-ring which removes the oxygen atom from the vicinity of the 7α-proton and so it moves upfield. The same conformational change takes 5′-H close to the C-4′ carbonyl and this is evidenced by the large downfield shift of 0.82 p.p.m. observed for 5′-H in dehydroaustin compared with austin itself. In addition 11-H shows an upfield shift of 0.40 p.p.m. in dehydroaustin consistent with it no longer being allylic. Final confirmation of the structure comes from the long range ^1H–^{13}C couplings observed in dehydroaustin. Again these have been extensively analysed by decoupling experiments and the relationships revealed are summarised in Figure 2(*b*). With the exceptions noted below these are essentially identical with those observed in austin. The significant differences are: (a) C-6′ now gives a pentet, which simplifies to either a quartet or doublet on irradiation of the 5′- or 10′-protons; (b) C-4′ again appears as a doublet, but now the splitting is due to a 3-bond coupling of 5 Hz to 5′-H and is removed on irradiation of 5′-H; and (c) C-9 appears as a broad multiplet which sharpens on irradiation of either 11-H or the 13-CH_2 protons. The methyl signals in the ^1H and ^{13}C spectra were again correlated by a Feeney plot.

The ^1H and ^{13}C n.m.r. spectra and molecular formula of compound (5) suggested that it was related to austin by loss of the 11-acetyl group. In the ^1H n.m.r. spectrum, 11-H appears at higher field than in austin as a doublet (5.5 Hz) at 4.59 p.p.m. coupled to a hydroxy-proton at 5.77 p.p.m. which exchanges with D_2O with the removal of the doublet splitting on 11-H. Confirmation of the structure was given by conversion of compound (5) into austin on acetylation with acetic anhydride and pyridine.

We have been engaged in a study of polyketide-derived metabolites of *Penicillium diversum*. In the course of this work we isolated from the culture liquors two metabolites which were obviously related to austin. The major of the two metabolites was clearly identical with austinol isolated from *A. ustus*. The minor metabolite had the same molecular formula $C_{27}H_{32}O_9$, and very similar ^1H and ^{13}C n.m.r. spectra, to austin. The p.n.d. ^{13}C n.m.r. spectrum shows 27 resonances with very similar chemical shifts and multiplicities to austin, the only significant differences being replacement of the doublet and triplet resonances at 74.7 and 27.0 p.p.m. assigned to C-11 and C-6, respectively, in austin by a doublet at 70.3 p.p.m. and a triplet at 42.0 p.p.m. The ^1H n.m.r. spectrum is particularly revealing. As discussed above, the 7α-proton in austin appears as a triplet of doublets at the unusually low field of 3.21 p.p.m. due to deshielding by the 6′-hydroxy-group. In the new metabolite, isoaustin, the 7α-proton appears as a triplet (*J* 13.2 Hz) which is coupled to doublets of doublets at 1.94 p.p.m. (*J* 13.2 and 3.9 Hz) and 4.89 p.p.m. (*J* 13.1 and 3.9 Hz) which can thus be assigned to the 7β-and 6β-protons respectively, and moreover the chemical shift of the 6β-proton indicates that the acetoxy-group must occupy the 6α-position. The 13-methyl protons now appear at 1.66 p.p.m. as a doublet (*J* 1.6 Hz) showing a homo-allylic coupling to the higher field proton of two mutually coupled (*J* 16.4 Hz) protons at 2.38 and 3.18 p.p.m. which must therefore be assigned to the 11-methylene. The 11α-proton in austin does not show any coupling to the 13-methyl so it must be the 11β-proton which is coupled in isoaustin. Dreiding models indicate that the 11α- and 11β-protons occupy pseudo-equatorial and pseudo-axial positions respectively and the angular

dependence for allylic coupling requires the proton to be approximately perpendicular to the plane of the olefinic system.[9] Confirmation of the structure again comes from the close similarity of the couplings in the fully ^{1}H-coupled ^{13}C n.m.r. spectrum to those observed for austin, the only observable differences being fully consistent with structure (6) for isoaustin. Thus the doublet of quartets due to C-11 in austin is replaced by a doublet (J 150 Hz) of double doublets (J 7 and 5 Hz) due to C-6, coupling presumably to the adjacent 7α- and 7β-protons. C-1, Which appears as a doublet of double doublets in austin due to 3-bond coupling to the 6-methylene protons, is simplified to a doublet of doublets (J 165 and 6 Hz) in isoaustin, the long range coupling being removed on irradiation of the 6β-proton at 4.89 p.p.m. Shortage of material precluded further decoupling experiments but all the remaining signals showed the expected coupling patterns.

The isolation of these closely related substances from a number of different organisms requires comment. We have recently shown, on the basis of ^{13}C-labelling studies, that austin and andibenin are probably formed *via* a common intermediate (7), itself formed by alkylation of 3,5-dimethylorsellinate by farnesyl pyrophosphate.[7] Thus the co-occurrence of andibenin B and austin in *A. variecolor* lends extra support to this proposal. In addition, the occurrence of related metabolites in *P. diversum* and terretonin in *A. terreus* indicates that this



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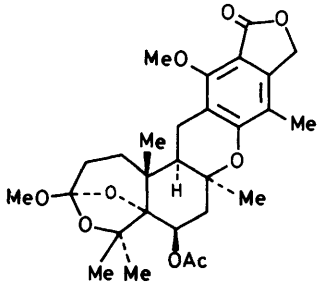

(8)

biosynthetic pathway is of relatively wide occurrence.* The recent isolation from *A. ustus* of the austalides, *e.g.* (8), which are clearly biogenetically related to the above compounds, should also be noted.[10]

EXPERIMENTAL

For general experimental details, see part 2.[1]

^{13}C *N.m.r. Determinations.*—Proton noise-decoupled and single frequency off-resonance decoupled spectra were determined on a Bruker WH 360 spectrometer operating at 90.56 MHz. Fully coupled spectra were determined under gated decoupling conditions to retain nuclear Overhauser effects. Specific ^{1}H-decoupling experiments were carried out using a decoupling power of 30 dB below 0.2 W. Decoupling frequencies were determined by observation of the ^{1}H n.m.r. spectrum using the decoupler coil of the ^{13}C probe for ^{1}H observation.

Isolation of Metabolites.—*Aspergillus ustus* (NRRL 6017) was grown at 25 °C in static culture in penicillin flasks each containing 200 ml of an aqueous medium made up from Oxoid malt extract (30 g l^{-1}) and Oxoid mycological peptone (10 g l^{-1}) in distilled water, autoclaved at 15 lb in^{-2} for 20 min, pH 5.4. After 14 d of growth the aqueous medium was separated from the mycelium and extracted with ethyl acetate. The resulting crude extract (350 mg l^{-1}) was purified by preparative t.l.c. using 4% methanol in chloroform as the eluting solvent. Removal of the u.v. quenching band at R_F 0.5—0.6 gave austin (110 mg l^{-1}), m.p. 300 °C (lit.,[4] 298—300 °C). Removal of the band at R_F 0.2—0.3 gave austinol (5) (35 mg l^{-1}). On recrystallisation from chloroform austinol had m.p. 320 °C (decomp.); $\lambda_{max.}$ (EtOH) 246 nm (ε 8 080); $\nu_{max.}$ (Nujol) 3 460, 3 350, 1 770, 1 720, and 1 680 cm^{-1} (Found: C, 65.6; H, 6.4. $C_{25}H_{30}O_7$ requires C, 65.49; H, 6.60%). Removal of the band at R_F 0.7—0.8 gave *dehydroaustin* (4) (30 mg l^{-1}) which on recrystallisation from ethanol had m.p. 284—286 °C; $\lambda_{infl.}$ (EtOH) 239 nm ($\varepsilon_{infl.}$ 3 800) and end absorption; $\nu_{max.}$ (KBr) 1 765, 1 755, and 1 710 cm^{-1} (Found: C, 64.9; H, 6.3. $C_{27}H_{36}O_9$ requires C, 65.1; H, 6.1%); $[\alpha]_D^{22}$ +127° (*c* 1.25%).

Penicillium diversum (ACC 946) was grown at 25 °C in static culture in penicillin flasks each containing Raulin-Thom medium (500 ml). The mycelium and liquors were separated and the liquors (50 l) were extracted with one third of their volume of chloroform. The chloroform extract was divided into acidic and neutral-phenolic fractions. The latter, on concentration, deposited crystals of austinol (5) (180 mg). On recrystallisation from chloroform austinol had m.p. 320 °C (decomp.); The mother liquors from above were subjected to preparative layer chromatography on 20 × 20 cm × 1 mm silica GF_{254} plates eluted with 4% ethanol in chloroform. Removal of the band with R_F 0.35 gave *isoaustin* (6) (15 mg), m.p. 290 °C (decomp.); $\lambda_{max.}$ (EtOH) 240 nm (ε 7 300); $\nu_{max.}$ (Nujol) 3 360, 1 765, 1 718, and 1 690 cm^{-1} (Found: C, 64.6; H, 6.4. $C_{27}H_{32}O_9$ requires C, 64.79; H, 6.44%); $[\alpha]_D^{22}$ +172.3° (*c*. 0.35%).

Aspergillus variecolor. An unidentified, variant strain of *A variecolour* was grown, as detailed above, on Czapek-Dox medium (50 pans). The mycelium and liquors (25 l) (pH 8) were separated by filtration, the liquors concentrated under reduced pressure to *ca.* 3 l and extracted with ethyl acetate (8 × 500 ml) to give an amorphous, brown solid (1.80 g) which was applied to 20 t.l.c. plates and developed in chloroform–methanol (98 : 2 v/v). The strongly u.v.-absorbing band at R_F 0.51 was removed and eluted with ethyl acetate to give dehydroaustin which recrystallised from ethanol as needles (60 mg), m.p. 284—286 °C. The strongly u.v.-absorbing band at R_F 0.34 was removed and eluted to give austin which recrystallised from methanol as stout needles (196 mg), m.p. 298—300 °C (lit.,[4] 298—300 °C) (Found: C, 64.9; H, 6.4. Calc. for $C_{27}H_{32}O_9$: C, 64.8; H, 6.4%); $[\alpha]_D^{22}$ +309° (*c* 0.98%).

Acetylation of Austinol.—Austinol (30 mg) was treated with acetic anhydride (0.5 ml) in pyridine (1.0 ml). Work-up gave austin (25 mg), m.p. and mixed m.p. 300—301 °C.

[2/512 *Received*, 24*th March*, 1982]

* *Note added in proof.* Austinol (5) has recently been isolated from *Emericella dentata* (Prof. Y. Maebayashi, personal communication).

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