someric 4-butyl-5,5-diethyl-2,2-dimethyl-3-decanols (**18a**) and 8.4 g of unchanged ketone: bp 118 °C (0.5 mm); n^{25}_D 1.4652; IR (neat) 3645 (s), 3583 (s), 3535 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.87 (m), 0.90–0.93 (s), 0.96–0.98 (s), 1.20–1.23 (s), 1.24–1.64 (m), 3.93–3.41 (d), 3.42–3.44 (s); ^{13}C NMR ($CDCl_3$) δ 84.7, 44.8, 43.6, 36.0, 35.1, 33.3, 33.2, 33.1, 28.2, 27.2, 27.0, 26.9, 24.7, 24.5, 23.8, 23.8, 23.7, 22.8, 14.2, 14.0, 9.4, 9.3, 8.7, 8.6. Two other secondary alcohols (**18b,c**) were prepared in the same manner.

2,2-Dimethyl-4,5,5-trialkyl-3-alkenes 19. The dehydration of the carbinols **18** was accomplished by heating them with $KHSO_4$ in essentially the same way as used for preparing alkenes **5**. Those obtained and their spectral properties are as follows: 4-butyl-5,5-diethyl-2,2-dimethyl-3-decene (**19a**) [IR (neat) 1625 (w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.04 (s, 1, C=CH), 2.00–2.5 (t, 3, allylic H's), 1.12 [s, 9, $C(CH_3)_3$], 0.64–0.95 (m, 30, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 140.7, 136.4, 45.4, 36.5, 34.0, 32.8, 31.8, 30.8, 28.8, 27.5, 27.0, 23.9, 23.3, 22.8, 14.2, 14.1, 8.3]; 3-*tert*-butyl-2,2-dimethyl-4,5,5-tripropyl-3-undecene (**19b**) [IR (neat) 1635 (w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.01 (s, 1, =CH), 1.95–2.00 (t, 2, allylic H's), 1.16–1.40 (m, 12, CH_2), 1.11 [s, 9, $C(CH_3)_3$], 0.82–1.17 (m, 20, CH_2, CH_3); ^{13}C NMR ($CDCl_3$) δ 141.6, 136.0, 45.5, 38.3, 35.8, 33.5, 32.2, 31.9, 30.5, 30.2, 25.3, 23.9, 23.0, 17.2, 15.2, 15.1, 14.3]; 3-*tert*-butyl-2,2-dimethyl-5,5-dipropyl-4-isopropyl-3-undecene (**19c**) [IR (neat) 1620 (w) cm^{-1}; UV (hexane) λ_{max} 200.5 nm (ϵ 8923); ^1H NMR ($CDCl_3$) δ 5.17 (s, 1, =CH), 2.04–2.07 (d, 2, allylic), 1.96–2.00 (m, 1, CH), 1.23–1.36 (m, CH_2), 1.12 [s, 9, $C(CH_3)_3$], 0.83–0.97 (m, CH_2, CH_3); ^{13}C NMR ($CDCl_3$) δ 139.1, 138.0, 45.5, 38.8, 36.9, 36.3, 32.2, 32.1, 30.4, 28.1, 23.8, 23.0, 22.8, 17.1, 15.0, 14.2; H–C long-range correlations (C=CH) irradiated; δ 139.06 (q, J_{CH} = 2.4 Hz), 45.43 (q, J_{CH} = 5.8 Hz), 36.93 (CH_2, J = 10.1 Hz), 33.16 (q, J = 2.1 Hz), 32.23 (CH_3, J = 3.9 Hz), 28.11 (CH, $J \cong 1$ Hz).

NMR Procedures. The 2-D INAPT sequence shown in Figure 2 is similar to Nagayama's modification[35] of the 1-D selective refocused INEPT experiment,[36] except that all of the proton pulses were soft, $\gamma(H_2)$ = 25 Hz giving a 90° proton pulse width of 10 ms. This modification was necessary on the Nicolet NT series of spectrometers which only allow rapid switching of the decoupler power between two levels. The F_1 spectral window was equal to $^1/_2t$ and was set to 40 Hz in this case. The delay Δ was optimized for J_{CH} = 7 Hz, and a 32 × 2K data set was acquired in approximately 2 h. This was processed with standard Nicolet software to yield a 64 × 1K frequency domain spectrum with DM = 4 and DM = 3 in the t_2 and t_1 dimensions, respectively.

The 2-D autocorrelated double quantum coherence spectroscopy experiment was performed ca. 500 mg of **19c** in a 5-mm carbon probe. A 64 × 4K data set was acquired in 47 h and processed to yield a 256 × 2K frequency domain spectrum. This experiment was optimized for J_{CC} = 42 Hz, and 12 correlations were observed.

(35) Jippo, T.; Kamo, O.; Nagayama, K. *J. Magn. Reson.* **1986**, *66*, 344–348.
(36) Bax, A.; Niu, C.-H.; Live, D. *J. Am. Chem. Soc.* **1984**, *106*, 1150–1151.

Revised Structures of Robustadials A and B from *Eucalyptus robusta*[1]

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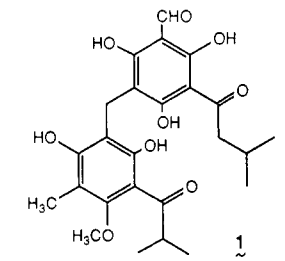
Received March 8, 1988

Robustadials A and B have been isolated from the leaves of *Eucalyptus robusta* Smith (Myrtaceae), separated as their dimethyl ethers, and their structures reinvestigated by spectroscopic techniques. The structures are shown to include the pinane skeleton by using various one- and two-dimensional NMR methods. Particularly useful were the 2D relayed coherence transfer and XCORFE pulse sequences.

Introduction

The Chinese traditional medicine "Da Ye An", prepared from the leaves of *Eucalyptus robusta* Smith (Myrtaceae), has a long, successful history for treating malaria and other ailments.[2] Activity against the malaria-inducing protozoan *Plasmodium berghei* has been traced to the phenolic components of the 95% ethanol extract, from which an active compound, robustaol A (**1**), was subsequently isolated.[3] A mixture of four chromans with even greater activity yielded two new compounds, robustadials A and B, which were assigned structures **2** and **3**.[4] The separation of **2** and **3** was accomplished after conversion to their dimethyl ethers. The remaining two chromans were identical with euglobals Ia_1 (**4**) and Ia_2 (**5**), previously reported from *Eucalyptus globulus*.[5]

Recently, the synthesis of **6**, the dimethyl ether of **2**, revealed that the structures of the robustadials were misassigned.[6] A new structure (**7**) was then suggested for robustadial A, robustadial B being the 7-epimer.[7] We now report the reisolation of robustadials A and B as their



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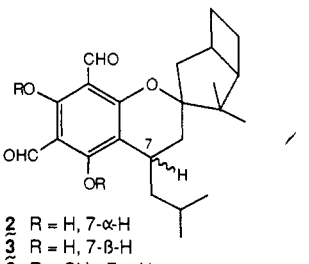
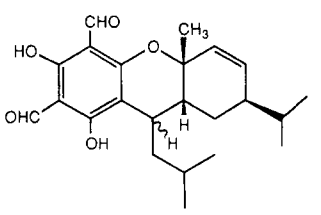
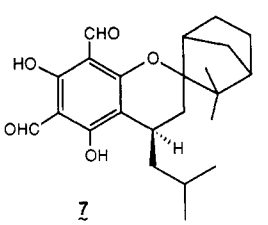


2 R = H, 7-α-H
3 R = H, 7-β-H
6 R = CH_3, 7-α-H



4 7-α-H
5 7-β-H



7

(1) This paper is dedicated to Professor Herman Kalckar on the occasion of his 80th birthday (March 26, 1988).
(2) In *Zhong Cao Yao Xue* (Nanjing Yao Xue Yuan); 'Zhong Cao Yao Xue' Bian Xie Zu, Jiang Su Ren Min Chu Ban She: Nanjing; Part 3, p 705.
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(4) Xu, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1984**, *106*, 734.
(5) Kozuka, M.; Sawada, T.; Kasahara, F.; Mizuta, E.; Amano, T.; Komiya, T.; Goto, M. *Chem. Pharm. Bull.* **1982**, *30*, 1952.
(6) Lal, K.; Zarate, E. A.; Youngs, W. J.; Salomon, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 1311.
(7) The numbering system is that proposed by Kozuka et al., ref 5.

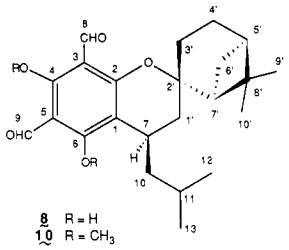
0022-3263/88/1953-4562$01.50/0

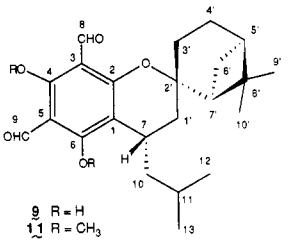
Table I. ^1H and ^{13}C NMR Data for 10 and 11, 93.94 kG, in $CDCl_3$[a]

position	^1H		^{13}C	
	10	11	10	11
1			118.8 (s)	118.8 (s)
2			163.5 (s)	163.4 (s)
3			115.3 (s)	115.4 (s)
4			164.9 (s)	165.7 (s)
5			116.1 (s)	116.4 (s)
6			165.4 (s)	165.5 (s)
7	2.93 (dddd, 10.3, 8.1, 7.3, 4.1)	2.97 (dddd, 10.2, 7.1, 6.9, 3.5)	27.1 (d)	26.9 (d)
8	10.38 (s)	10.34 (s)	187.6 (d)	187.6 (d)
9	10.30 (s)	10.26 (s)	187.6 (d)	187.6 (d)
10	1.26 (ddd, 13.1, 10.2, 4.1)	1.27 (ddd, 13.0, 9.8, 3.5)	44.0 (t)	44.5 (t)
	1.87 (ddd, 13.1, 10.3, 3.6)	1.91 (ddd, 13.0, 10.2, 2.5)		
11	1.70 (qqdd, 6.4, 6.4, 10.2, 3.6)	1.72 (qqdd, 6.5, 6.5, 9.8, 3.5)	25.5 (d)	25.8 (d)
12	0.95 (d, 6.4)	0.93 (d, 6.5)	21.0 (q)	21.0 (q)
13	1.00 (d, 6.4)	1.01 (d, 6.5)	24.0 (q)	23.8 (q)
1′	1.91 (dd, 14.3, 8.1, Ha)	1.77 (dd, 13.9, 6.9 Ha)	39.3 (t)	39.1 (t)
	2.19 (dd, 14.3, 7.3, Hb)	2.23 (dd, 13.9, 7.1, Hb)		
2′			85.7 (s)	84.8 (s)
3′	1.99 (m, Ha)	1.94 (m, 2 H)	30.9 (t)	28.2 (t)
	2.22 (m, Hb)			
4′	1.88 (m, Ha)	1.92 (m, 2 H)	24.8 (t)	24.8 (t)
	2.02 (m, Hb)			
5′	1.98 (m)	1.99 (m)	40.5 (d)	40.5 (d)
6′	1.53 (d, 9.4)	1.65 (d, 9.4)	26.6 (t)	27.0 (t)
	2.10 (m)	2.27 (m)		
7′	2.03 (m)	2.25 (m)	48.9 (d)	51.0 (d)
8′			38.2 (s)	38.2 (s)
9′	1.26 (s)	1.30 (s)	27.7 (q)	27.3 (q)
10′	1.03 (s)	1.04 (s)	23.4 (q)	23.4 (q)
4-OCH_3	3.96 (s)	3.94 (s)	64.9 (q)	64.8 (q)
6-OCH_3	3.88 (s)	3.87 (s)	62.5 (q)	62.5 (q)

[a] ^{13}C multiplicities assigned by DEPT; all one-bond heteronuclear couplings confirmed by HETCOR.

dimethyl ethers and show that their correct structures contain the pinane skeleton (**8** and **9** respectively) on the basis of spectroscopic techniques.





Results and Discussion

The oily robustadial epimers were isolated from the crude ethanolic extract and subsequently separated as their dimethyl ethers (**10** and **11**) as previously described (see Experimental Section).[4] Separation of the parent phenols, **8** and **9**, was extremely difficult.[8] Comparison of the ^1H and ^{13}C NMR spectra with those previously recorded[4] confirmed the identity of these chroman ethers, Table I. The fully substituted aromatic ring of the phloroglucinol-derived moiety, common to *Eucalyptus* spp.,[3,5,9] was readily apparent from the NMR (Table I), UV, and IR spectroscopic data.[4] The connectivity from the isobutyl chain through the benzylic (H-7) proton to the C-1′ methylene group was clearly mapped with a double quantum filtered, phase sensitive COSY spectrum (DQCOSY), supported by the fragmentation pattern in the mass spectrum.[4,5] Difference decoupling experiments (particularly decoupling of H-7 and H-11) confirmed the DQCOSY coupling network. A HETCOR spectrum subsequently enabled assignment of C-7 and C-10 through C-13.

The main difficulty in assigning the structures of **10** and **11** was deducing the nature of the hydrocarbon unit attached to the spiral center (C-2′). From the molecular formula (HRMS, **10**: m/z 414.2416 [M^+], calcd for $C_{25}H_{34}O_5$ 414.2406), it was clear that nine carbons, including the spiral center, arranged in a bicyclic skeleton (two units of unsaturation) were required. Initial work focused on **10**.

A DEPT spectrum required that the nine remaining carbons of the bicyclic unit to be two methyls, three methylenes, two methines, and two quaternary carbons. Mutual three-bond heteronuclear couplings (XCORFE)[10] between the two methyl groups (δ 1.03, 1.26, both s, one bond coupled to ^{13}C δ 23.4 and 27.7, respectively, from HETCOR) confirmed the presence of a *gem*-dimethyl unit (accounting for the two methyls and the higher field quaternary carbon, δ 38.2 of the bicyclic unit), requiring the two methine carbons to be bridgeheads and the C-2′ quaternary carbon (δ 85.7, s) to be a spiral center. This information rules out possible structures such as A with a methyl group located at C-2′. Similarly, three-bond heteronuclear couplings (XCORFE) between both methyl groups and the two bridgehead methines were also detected, thereby requiring that the *gem*-dimethyl group be flanked by the bridgeheads and thus ruling out the previously proposed camphane-bearing structure **7**.[16] Since the $^1J_{C-H}$ coupling constants of the methine bridgeheads,

(8) Conditions described for the separation of the related euglobals were not successful in resolving **8** and **9**: Amano, T.; Komiya, T.; Hori, M.; Goto, M.; Kozuka, M.; Sawada, T. *J. Chromatogr.* **1981**, *208*, 347.

(9) (a) Crow, W. D.; Osawa, T.; Paton, D. M.; Willing, R. R. *Tetrahedron Lett.* **1977**, 1073. (b) Sawada, T.; Kozuka, M.; Komiya, T.; Amano, T.; Goto, M. *Chem. Pharm. Bull.* **1980**, *28*, 2546. (c) Kozuka, M.; Sawada, T.; Kasahara, F.; Mizuta, E.; Amano, T.; Komiya, T.; Goto, M. *Chem. Pharm. Bull.* **1982**, *30*, 1964. (d) Qin, G. W.; Xu, R. *Huaxue Xuebao* **1986**, *44*, 151.

(10) Reynolds, W. F.; Hughes, D. W.; Perpick-Dumont, M. *J. Magn. Reson.* **1985**, *63*, 413.

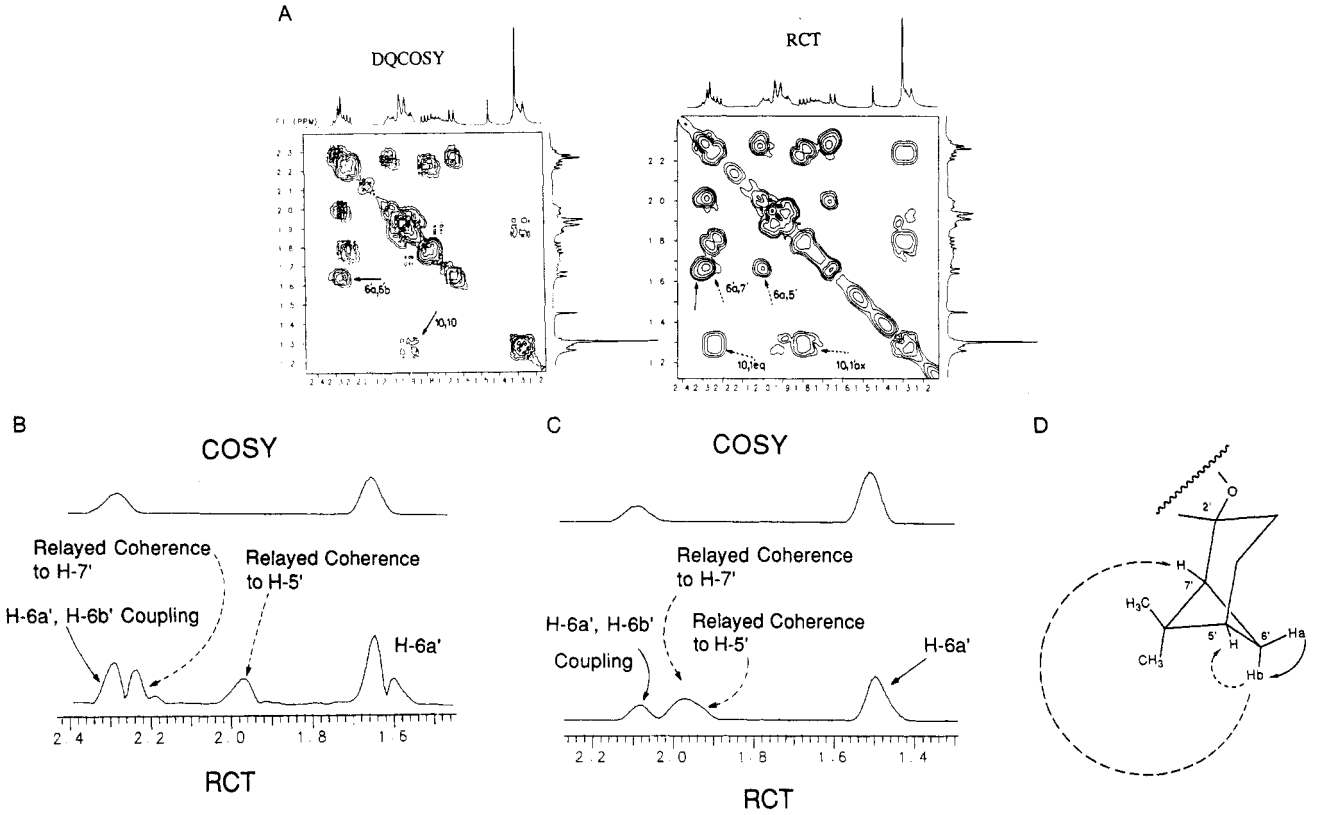
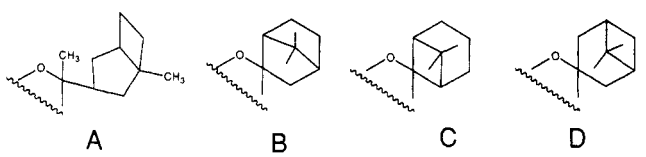


Figure 1. (A) Relayed coherence transfer (RCT) and double quantum filtered, phase sensitive COSY (DQCOSY) spectra of **11**. Direct coherence transfer is indicated by solid arrows, relayed coherence by dashed arrows. (B) Cross sections at δ 1.646 (H-6a') of **11**. RCT cross section shows magnetization from H-6a' relayed through H-6b' to the two bridgehead protons, H-5' and H-7', compared to COSY spectrum at same frequency. (C) Cross section in RCT spectrum of **10** at δ 1.534 (H-6a') compared to coherence in COSY spectrum at same frequency. Overlap in the signals from H-5' and H-7' of **10** prevents resolution of relayed coherence to these two protons. (D) Coherence between H-6a' and H-6b' (solid arrow) relayed to H-5' and H-7' (dashed arrow) in pinane unit of **10** and **11**.

δ 48.9 (d, 145.7 Hz, one bond coupled to ^{1}H δ 2.03, m, from HETCOR) and 40.5 (d, 141.2 Hz, one bond coupled to ^{1}H δ 1.98, m, from HETCOR), precluded the possibility of a cyclopropane ring, either a [3.1.1] or [2.2.1] bicyclic system was deduced.



Biosynthetically, the [3.1.1]-bicyclic pinane system is the most attractive for the structure. Nonetheless, other alternatives with the *gem*-dimethyl unit flanked by bridgehead methines, such as potential substructures B–D, had to be considered. A conspicuous doublet (δ 1.53, 9.4 Hz, H-6a'), characteristic of the pinane skeleton, showed coupling (DQCOSY) only to its gem partner, δ 2.10 (m, H-6b', gem relationship confirmed by HETCOR, both one bond coupled to the same carbon, ^{13}C δ 26.6). This gem proton appeared to be coupled to both bridgehead methines, δ 1.98 (m, H-5') and 2.03 (m, H-7'), but these couplings were difficult to confirm in the DQCOSY spectrum of **10** due to the proximity of the chemical shifts. Thus, the cross peaks in the spectrum were too close to the diagonal to be unambiguously assigned. The signal congestion in this region of the spectrum of **11**, however, was sufficiently reduced, and H-6b' (2.27, m), H-7' (2.25, m), and H-5' (1.99, m) could be distinguished and the vicinal coupling from H-6b' to both bridgehead protons confirmed. The HETCOR experiment thus allowed for assignment of C-6' (27.0, t), C-7' (51.0, d), and C-5' (40.5, d) for **11**.

A 2D relayed coherence transfer spectrum (RCT)[11] on **11** further established that the methylene bearing the doublet is flanked by the bridgehead methines. Thus, coherence between the gem protons (C-6' methylene) was relayed to both methines, Figure 1. An analogous RCT on **10** also indicated relayed coherence from H-6a' through H-6b' to both methines (H-5' and H-7'), but overlap prevented unambiguous assignment, Figure 1C. Such a spin system eliminates substructures B and C as possibilities. Subunit D was ruled out by the observation of three-bond heteronuclear coupling (XCORFE) between the C-6' protons and the spiral center carbon. Three-bond coupling from the H-6a' doublet to the C-8' quaternary carbon bearing the *gem*-dimethyl unit observed in the XCORFE spectrum also confirmed the cyclobutane portion of the pinane subunit.

Three-bond heteronuclear couplings in **10** which traversed the spiral center at C-2' further confirmed the pinane subunit. Thus, couplings to C-1' (δ 39.3) from both protons of a methylene group (H-3': δ 1.99 and 2.22, one bond coupled to ^{13}C δ 30.9 from HETCOR) and the methine proton (H-7': δ 2.03) were detailed in the XCORFE cross sections, Figure 2. The key point of these couplings is that they require a methylene and a methine carbon to be adjacent to the spiral center in the bicyclic monoterpene unit. As described above, the methylene unit which bears the conspicuous doublet at δ 1.53 (C-6') and the *gem*-dimethyl unit are flanked by methines. These observations required structure E, which lacks only the placement of the final carbon, a methylene (δ 24.8, one bond coupled

(11) (a) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3732. (b) Bax, A.; Drobmy, G. *J. Magn. Reson.* **1985**, *61*, 306.

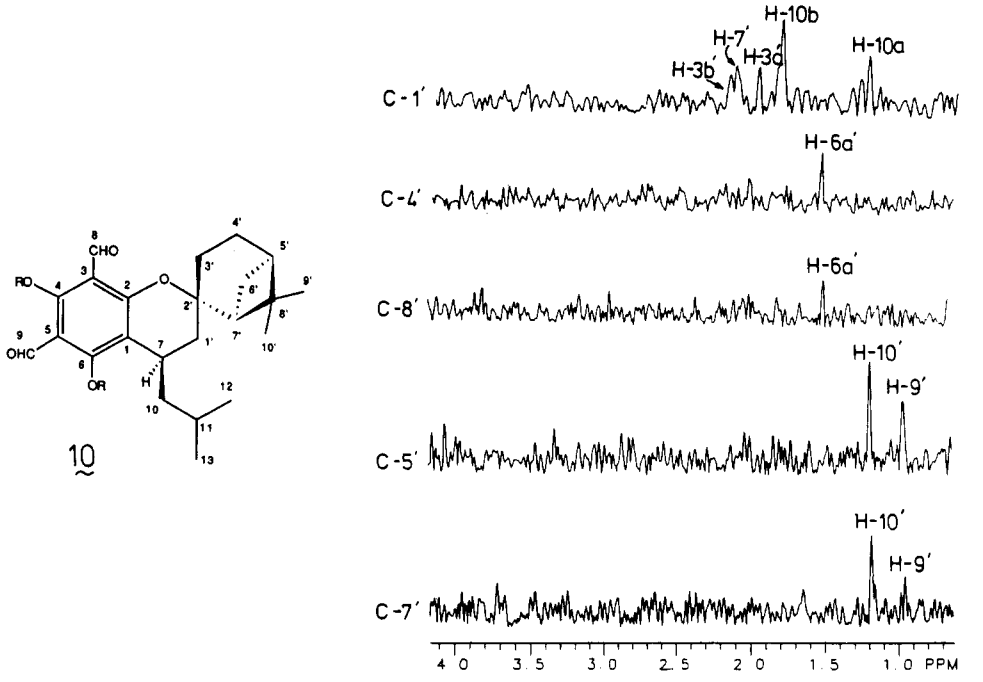
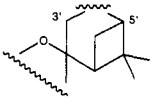


Figure 2. Cross sections from the XCORFE spectrum of **10** taken at the frequencies of the indicated carbons.

Table II. Long-Range Heteronuclear ($^3J_{CH}$ and $^2J_{CH}$) Couplings in 10 Detected by XCORFE and Long-Range HETCOR

carbon	proton(s)	experiment
2	7	XCORFE
3	8	HETCOR
4	OCH_3	XCORFE
5	9	HETCOR
6	OCH_3	XCORFE
1′	10a, 10b, 3a′, 3b′, 7′	XCORFE
2′	6a′, 6ba	XCORFE
4′	6a′	XCORFE
5′	9′, 10′	XCORFE
7′	9′,10′	XCORFE
8′	6a′	XCORFE
9′	10′	XCORFE
10′	9′	XCORFE

to ^{1}H δ 1.88, m, and 2.02, m, from HETCOR). Clearly this methylene must be the carbon that links C-3′ and C-5′. The only possible structure that follows these observations is the [3.1.1]-bicyclic skeleton of pinane. Three-bond coupling from the H-6a′ proton to C-4′ confirms this conclusion.



E

With the observed heteronuclear couplings detected in the HETCOR and XCORFE spectra, assignment of all ^{13}C NMR signals of **10** was possible, Table I. The methylene (δ 24.8) and methine (δ 40.5) carbons which were not adjacent to the spiral center of the pinane unit in the DEPT spectrum must be assigned to C-4′ and C-5′, respectively. Cross sections from the HETCOR and XCORFE spectra allowed for assignment of chemical shift frequencies for all protons and carbons, Table I. The crucial cross sections from the XCORFE spectrum are shown in Figure 2. Table II summarizes the long-range heteronuclear couplings used in assigning the pinane skeleton signals.

With the basic carbon skeleton resolved, it remained to establish the relative stereochemistry of the pinane unit with respect to the benzylic carbon, C-7. This task was accomplished with 2D NOE and difference NOE spectra. For both **10** and **11**, NOEs between the C-8 aldehyde proton and the H-6a′ doublet require the relative stereochemistry of the pinane unit as in **8**–**11** (rather than the reversal of the methylene and *gem*-dimethyl groups of the cyclobutane ring). Confirmation of **10** as the α-H-7 epimer (H-7 and H-7′ in a syn relationship relative to the B ring) was apparent from the NOE between the benzylic proton (H-7) and H-7′, and NOE between the 10′-CH_3 and the pseudoequatorial H-1b′. Other NOEs and coupling constants are in accord with this structure and the ^{1}H and ^{13}C assignments, Figure 3.

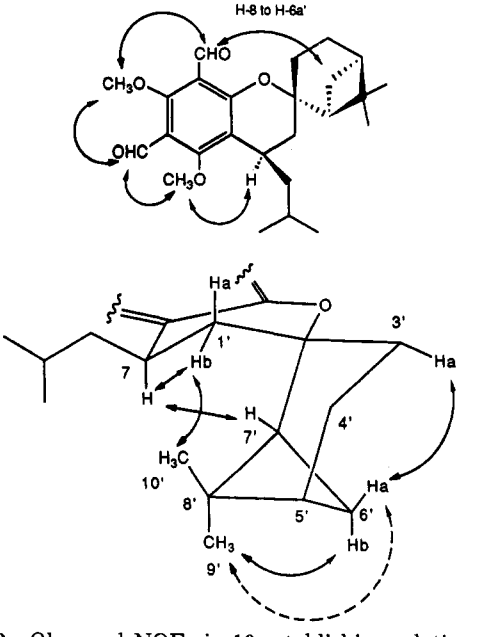


Figure 3. Observed NOEs in **10** establishing relative stereochemistry. Conformations drawn are also in agreement with observed coupling constants between H-7 to H-1′ methylene protons (Table I). Dashed arrows indicate negative NOE.

Identification of dimethylrobustadial B as the β-H-7 epimer, **11**, was routine. The ^{1}H and ^{13}C NMR spectra

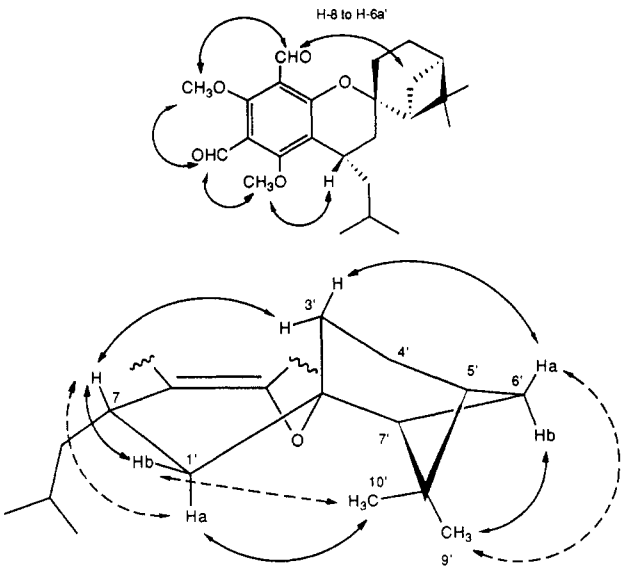


Figure 4. Observed NOEs in **11** establishing relative stereochemistry. Conformations drawn are also in agreement with observed coupling constants between H-7 to H-1′ methylene protons (Table I). Dashed arrows indicate negative NOE.

were strikingly similar to those of **10**, Table I. Spectroscopic analysis as described above for **10** confirmed the identity of the carbon skeleton. The relative stereochemistry was established by difference and 2D NOE spectra, Figure 4. Thus, NOEs were observed between the H-7 and H-3′ protons,[12] and between the 10′-CH_3 and the pseudoequatorial H-1b′. This latter NOE was supported by a negative NOE from the 10′-CH_3 to H-1a′. The absolute stereochemistry of **10** and **11** is as yet undetermined.

Assignment of Aromatic Carbons and Substituents. The ^1H signals of the two methoxy methyl and two aldehyde protons of both **10** and **11** were readily distinguished by the 2D NOE spectrum, Figures 3 and 4. Thus, the methoxyl group (**10**, δ 3.88; **11**, δ 3.87) with an NOE to the benzylic H-7 and one of the two aldehyde protons (**10**, δ 10.30; **11**, δ 10.26) is located at C-6 of the aromatic nucleus. The other methoxyl group (**10**, δ 3.96; **11**, δ 3.94) with NOEs to both aldehyde protons must be located at C-4. These NOEs also distinguish the two aldehyde protons; one (H-9, **10**, δ 10.30; **11**, δ 10.26) with NOEs to both methoxyl groups is located at C-5 of the aromatic ring while the aldehyde proton (H-8, **10**, δ 10.38; **11**, δ 10.34) with an NOE to only one methoxyl group (as well as the very important NOE to H-6a′) must be located at C-3. The assignment of the proton signals of the methoxyl substituents also enabled the ^{13}C NMR assignments of their respective methyl groups (Table I).

With the proton signals of these substituents assigned, assignments of the aromatic carbons, C-1 through C-6, were completed for **10** and **11** by the observation of two- and three-bond heteronuclear couplings, confirmed by long-range HETCOR, XCORFE, and selective population transfer (SPT),[13] also summarized in Table II. Three-bond coupling between the methoxyl protons and aromatic carbon at the methoxyl substitution site (observed by both XCORFE and SPT) enabled the assignment of C-4 (**10**, δ 164.9 coupled with ^1H δ 3.96; **11**, δ 165.7 coupled with ^1H δ 3.94). Likewise, C-6 was assigned by three-bond coupling to the methoxyl protons which displayed an NOE to H-7 (**10**, δ 165.4 coupled with ^1H δ 3.88; **11**, δ 165.5 coupled with ^1H δ 3.87).

The characteristically large two-bond heteronuclear coupling of aldehyde protons[14] enabled the assignment of C-3 and C-5. Thus, coupling was observed between H-8 and C-3 (**10**, δ 115.3, d, $^2J_{C-H}$ = 23 Hz, coupled with ^1H δ 10.38; **11**, δ 115.4, d, $^2J_{C-H}$ = 23 Hz, coupled with ^1H δ 10.34), as well as between H-9 and C-5 (**10**, δ 116.1, d, $^2J_{C-H}$ = 23 Hz, coupled with ^1H δ 10.30; **11**, δ 116.4, d, $^2J_{C-H}$ = 23 Hz, coupled with ^1H δ 10.26).

The remaining two aromatic carbons, C-1 (**10**, δ 118.8; **11**, δ 118.8) and C-2 (**10**, δ 163.5; **11**, δ 163.4) were easily distinguished on the basis of the chemical shifts. Long-range heteronuclear three-bond coupling observed in the XCORFE spectrum of **10** between H-7 and C-2 supported these already obvious assignments. Hence the observation of long-range heteronuclear couplings allows for the assignment of all the aromatic carbons without resorting to calculations based on additivity rules.

Further efforts to separate the parent robustadials, **8** and **9**, are continuing in order to assess their biological activity.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were recorded on a Varian XL-400 (93.94 kG, 400 MHz for ^1H, 100 MHz for ^{13}C) in $CDCl_3$. The 7.24 ppm resonance of residual $CHCl_3$ and 77.0 ppm resonance of $CDCl_3$ were used as internal references for ^1H and ^{13}C, respectively. All 2D pulse sequences were run by using standard Varian software, version 6.1C. ^1H resonance frequency and coupling constant determinations were accomplished via slices (cross sections) through the contour plot of the DQCOSY spectrum and confirmed by a 2D-J spectrum. The 2D-J spectra were helpful due to the spectral congestion. Thus, the linear alignment of the multiplets enabled resolution of signals that were overlapped in the DQCOSY spectra. Difference NOE spectra were recorded to complement the 2D NOE spectra since the difference technique allowed for more routine detection of negative NOEs and also enabled unambiguous assignment since the multiplicities of the enhanced signals were more readily discernible. ^{13}C multiplicities were assigned by using DEPT experiments, and carbon assignments were completed with HETCOR and XCORFE experiments. The RCT spectra of **10** and **11**, Figure 1, were recorded by using spectral windows of 1176 Hz and 1472 Hz in both dimensions for **11** and **10**, respectively, and acquisition times of 0.218 s for both compounds, with 256 increments, using 80 and 128 transients per increment, **11** and **10**, respectively; the intervals, τ, were set to 20 ms. The data were processed as 1024 × 1024 matrices for both compounds. The XCORFE spectrum of **10**, Figure 2, was recorded with spectral windows of 20 000 Hz (acquisition time of 0.102 s) in the carbon dimension and 1414 Hz in the proton dimension, with 198 increments, using 64 transients per increment. The BIRD pulse intervals, τ, were set to 4 ms, and the refocusing interval, $\Delta/2$, was set to 25 ms. The data were processed as a 4096 × 512 matrix. The 2D NOE spectrum of **10**, results shown in Figure 3, was recorded by using spectral windows of 5000 Hz in both dimensions (1024 points for each dimension) with 256 increments, using 64 transients per increment. The mixing time was set to 0.6 s. The 2D NOE spectrum of **11**, results shown in Figure 4, was run under conditions identical with those for **10**. Mass spectra were recorded on a Finnigan MAT-90 mass spectrometer (70 eV for EI).

Isolation of Robustadial Mixture. The leaves of *E. robusta* Smith (17 kg, dry wt), obtained from Guangzhou Medicinal Materials Company (Guangzhou Shi Yao Cai Gong Si), Guangzhou, People's Republic of China, were thoroughly extracted with 95% EtOH and filtered, and the extract was concentrated in vacuo (residue 1.25 kg). The extract was repeatedly chromatographed [flash silica gel; 50:1 petroleum ether (bp 30–60 °C)/EtOAc] to yield the phenolic, robustadial mixture (containing **4**, **5**, **8**, **9**, and small amounts of other phenolic material). The identity of the

(12) An NOE was also apparent from H-7 to H-4′, presumably the axial. Due to the proximity of the H-3′ and H-4′ signals, however, δ 1.94 (2 H, H-3′) and 1.92 (2 H, H-4′), it was difficult to distinguish this NOE. According to Dreiding models, both the H-3′ equatorial and H-4′ axial protons lie about 2.5 Å from H-7.

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(14) Ewing, D. F. *Org. Magn. Reson.* **1971**, *3*, 279.

mixture was confirmed by ^1H NMR.[15]

Methylation of Robustadials. Isolation of Dimethylrobustadial A (10) and Dimethylrobustadial B (11). To a solution of the robustadial mixture (100 mg) in anhydrous acetone (20 mL) were added CH_3I (150 mg, 4 molar equiv based on 8) and K_2CO_3 (500 mg). The reaction mixture was refluxed for 8 h with further addition of CH_3I (150 mg) every 2 h. After cooling, the mixture was filtered and the solvent removed in vacuo. The residue was chromatographed [flash silica gel; 5:1 petroleum ether (bp 30–60 °C)/EtOAc] to obtain partially purified fractions of **10**, **11**, and the dimethyl ethers of **4** and **5**. Final purification of **10** and **11** was by normal-phase HPLC [Microsorb (5 μm), Rainin, 4.6 × 250 mm; flow rate, 1.5 mL/min; UV detection, 254 nm; 0.03% *i*-PrOH in *n*-heptane].

Dimethylrobustadial A (10): colorless oil; HRMS, *m/e* 414.2416 [M$^+$], calcd for $C_{25}H_{34}O_5$ 414.2406; UV λ_{max} (MeOH) 261 (ε 11 500), 280 (9200), 320 nm (sh, 2880); CD (MeOH)[4] 235 (Δε +1.96), 259 (+3.86), 275 (+2.29), 320 (+1.81), 354 nm (−0.59); IR (NaCl, film) 2957, 2928, 2860, 1686, 1566, 1462, 1383, 1121, 1070 cm^{-1}; ^1H and ^{13}C NMR, see Table I; low-resolution MS, *m/z* (relative intensity) 414 (M$^+$, 0.8), 399 (4), 371 (2), 357 (11), 279 (21), 223 (100), 136 (2), 91 (45).

Dimethylrobustadial B (11): colorless oil; UV λ_{max} (MeOH) 261 (ε 11 500), 280 (9200), 320 nm (sh, 2880); CD (MeOH)[4] 227 (Δε −2.54), 257 (−2.50), 292 (+1.15), 330 (+0.47), 354 nm (+0.19); IR (NaCl, film) 2953, 2928, 2868, 1686, 1564, 1461, 1385, 1126, 1103 cm^{-1}; ^1H and ^{13}C NMR, see Table I; low-resolution MS, *m/z* (relative intensity) 414 (M$^+$, 3), 399 (6), 371 (10), 357 (19), 279 (55), 223 (100), 136 (3), 91 (47).

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Supplementary Material Available: DQCOSY, DEPT, and HETCOR spectra for **10** and HETCOR spectrum for **11** (11 pages). Ordering information is given on any current masthead page.

(15) Small amounts of the parent phenols, **8** and **9**, were separated and their high-resolution mass spectra reported in ref 4.

(16) While this manuscript was in press, the syntheses of robustadials A and B with the correct structures **4** and **5** were reported. Salomon, R. G.; Lal, K.; Mazza, S. M.; Zarate, E. A.; Youngs, W. J. *J. Am. Chem. Soc.* **1988**, *110*, 5213.

Podosporin A: A Novel Antifungal Metabolite from the Coprophilous Fungus *Podospora decipiens* (Wint.) Niessl

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Podosporin A (1), a new tetracyclic sesquiterpene quinone exhibiting antifungal activity, antibacterial activity, and brine shrimp toxicity, has been isolated from the coprophilous fungus *Podospora decipiens* (NRRL 6461), a colonist of cattle dung. Podosporin A was obtained from ethyl acetate extracts of liquid cultures of *P. decipiens* by silica gel chromatography and reversed-phase HPLC, and its structure was assigned by single-crystal X-ray diffraction analysis. The isolation process was guided by in vitro bioassays for antifungal antagonism toward other coprophilous fungi.

The phenomenon of interference competition among fungi from competitive ecological niches is commonly observed,[1-6] but the chemistry associated with these interactions remains largely unexplored. We have undertaken studies of such systems in order to obtain insight into the chemical ecology of interspecies competition, and to explore the potential value of these organisms as sources of natural antifungal agents.[1,2] During examination of the chemical basis for interspecies competition among coprophilous (dung-colonizing) fungi, we have encountered a novel antifungal metabolite produced by the late successional fungus *Podospora decipiens* (NRRL 6461), an ascomycete that exhibits antagonistic activity toward other coprophilous fungi in vitro. We now report details of the isolation, structure, and biological activity of this compound, which we have named podosporin A.

Table I. Proton NMR Assignments for Podosporin A (1)[a]

position		position	
2	6.47 (d, 1.2 Hz)	16	2.05 (br ddd, 13, 12, 4.2)
7	2.83 (d, 19)		1.42 (br dd, 13, 3.4)
	1.92 (d, 19)	17	1.74 (m)
10	2.21 (ddd, 14, 13,	18	1.08 (d, 7.3)
	3.9)	19	0.92 (s)[b]
	1.72 (m)	20	4.12 (dq, 7.1, 1.2)
11	2.50 (m)	22	6.16 (dq, 16, 1.7)
	1.63 (m)	23	6.94 (dq, 16, 6.8)
12	3.52 (br s)	24	1.88 (dd, 6.8, 1.7)
14	1.73 (m)	25	1.26 (d, 7.1)
15	1.88 (m)	26	0.87 (s)[b]
	1.32 (m)	27	0.90 (s)[b]

[a] Spectrum recorded at 360 MHz in $CDCl_3$. [b] Assignments are interchangeable.

Podosporin A (1) was obtained from ethyl acetate extracts of liquid cultures of *P. decipiens* by silica gel chromatography and reversed-phase HPLC. The isolation of this metabolite was guided by bioassays for antifungal activity toward the early successional dung fungi *Sordaria*

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