Notes

ISOLATION OF (−)-γ-CADINENE AND ARISTOLOCHENE FROM *ASPERGILLUS TERREUS*

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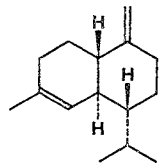
The fungus *Aspergillus terreus* is capable of biosynthesizing a remarkable number of natural products. Among the diverse metabolites which have been isolated from various strains of *A. terreus* are tetraketides: 3-methylorsellinate;[1] depside tetraketides: 4-*O*-demethylbarbatic acid;[2] pentaketides: terrein[3] and citrinin;[4] octaketides: questin,[5] sulochrin,[5] and dehydrocurvularin;† nonaketides: citreoviridin[6] and mevinolin;[7] triprenyltetraketides: terretonin;[8,9] tetraketide toluoquinones: terremutin hydrate;[10] shikimate-derived phenyl propanoids: aspulvinones;[11] indole propanoids: asterriquinone;[2] diketopiperazines: acetylaranotin[12] and astechrome;[13] anthranilates;[14] and sesquiterpenes: apterric acid,[15] quadrone[16] and terrecyclic acid.[17] In the course of our own work on the biosynthesis of quadrone and terrecyclic acid,[18,19] we have been examining the non-polar mycelial extracts of *A. terreus*. We now report the isolation of two sesquiterpene hydrocarbons, (−)-γ-cadinene (**1**) and aristolochene (**2**), neither of which has previously been reported as a fungal metabolite.

Seed cultures of *A. terreus* NRRL 11,156, grown for 2~3 days at 25°C as previously described,[19] were used to inoculate a 4-liter production culture (1:40). The fermentation was continued with aeration for periods of 48~160 hours, after which the mycelia was harvested by filtration and continuously extracted for 4 hours with acetone and 18 hours with pentane. The aqueous acetone extracts were further extracted with pentane and the combined pentane extracts were concentrated to 20 ml by distillation at atmospheric pressure using a Vigreux column. Filtration of the concentrate through silica gel and elution with pentane served to remove polar constituents. Analysis of the pentane eluent by capillary gas chromatography (GC) (25 m×0.2 mm×0.1 μm HP-20M Carbowax 20M; 110 ml/minute He; T(1)=40°C, 0.5 minute, ramp at 10°C/minute, T(2)=200°C) revealed the presence of three major components: **A** (retention time (Rt) 6.88 minutes), **B** (Rt 7.92 minutes) and **C** (Rt 8.58 minutes). The proportions of the individual components varied with incubation time, with **A** and **B** being favored by incubations of 48~72 hours and **C** predominating at 100~160 hours. GC-MS analysis indicated a MW of *m/z* 204 for each component, consistent with a sesquiterpene hydrocarbon, $C_{15}H_{24}$, having four units of unsaturation. The extracts were further concentrated to 0.5~1.0 ml, applied to a 13×1.25 cm column of TLC-grade silica gel, and eluted with pentane, fractions of 9 ml being collected. Compound **B** (TLC Rf 0.85, pentane) typically was found in Fractions 3 and 4, while **C** was located in Fractions 4~6, the latter fractions also containing small amounts of **A**. Where necessary, each component was further purified by a second silica gel column or argentation silica gel chromatography. Using these protocols, it was possible to accumulate 2~4 mg of **B** and 4~6 mg of **C**. **A**, which was recovered in only minor quantities, has not been investigated further.

High field ^{1}H (250 MHz) and ^{13}C (62.9 MHz) NMR analysis of **C** established the presence of an exomethylene double bond (^{1}H δ 4.55 and 4.66, ^{13}C δ 103.14 (t) and 153.35 (s)) and a trisubstituted double bond (^{1}H δ 5.55, ^{13}C δ 122.52 (d) and 134.68 (s)), indicating that **C** was bicyclic. Three methyl groups were evident (^{13}C δ 15.21 (q), 21.57 (q) and 23.85 (q)), consisting of a pair of isopropyl methyl doublets (^{1}H δ 0.74 (d, J=6.9 Hz) and 0.92 (d, J=6.9 Hz)) and an allylic methyl (^{1}H δ 1.69). The presence of four additional methylene carbons (^{13}C δ 25.81 (t), 26.69 (t), 30.61 (t) and 36.41 (t)) and four methines (^{13}C δ 26.32 (d), 44.32 (d), 45.27 (d) and 47.08 (d)) suggested that **C** was γ-cadinene or a

† Unpublished observations, D. E. CANE and B. J. RAWLINGS.



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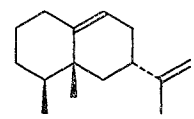

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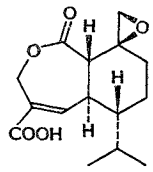

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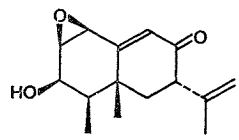

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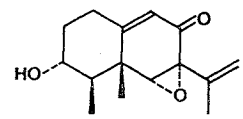

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stereoisomer. The structure of **C** was confirmed as (−)-γ-cadinene (**1**) by direct comparison with an authentic sample of (+)-γ-cadinene.† The two samples were identical by ^{1}H and ^{13}C NMR and capillary GC RT but showed opposite Cotton effects in the CD spectrum of a hexane solution. (−)-γ-Cadinene (**1**): $\Delta\varepsilon$ (203 nm) −16.6, $\Delta\varepsilon$ (194 nm) 0, $\Delta\varepsilon$ (188 nm) +9.7; (+)-γ-cadinene: $\Delta\varepsilon$ (204 nm) +18.0, $\Delta\varepsilon$ (194 nm) 0, $\Delta\varepsilon$ (188 nm) −10.[20,21]

The structure of component **B** was assigned as aristolochene (**2**)[22,23] by extensive ^{1}H and ^{13}C NMR analysis, followed by direct comparison with an authentic sample of synthetic (±)-aristolochene.[24] The bicyclic structure of **B** was inferred from the presence of two double bonds, an exomethylene (^{1}H δ 4.69 (2H), ^{13}C δ 108.28 (t) and 150.57 (s)) and a tri-substituted olefinic bond (^{1}H δ 5.29 (dt, J=1.9 and 5.4 Hz), ^{13}C δ 118.79 (d) and 144.49 (s)). A secondary methyl group (^{1}H δ 0.83, ^{13}C δ 15.68 (q)), a methyl group attached to a quaternary carbon (^{1}H δ 0.95, ^{13}C δ 18.13 (q)), and an allylic methyl (^{1}H δ 1.72, ^{13}C δ 20.84 (q)) were readily recognizable. Detailed analysis of ^{1}H-^{1}H homonuclear correlation spectroscopy (HOMOCOSY), ^{1}H nuclear Overhauser effect spectroscopy (NOESY), and ^{1}H-^{13}C heteronuclear correlation spectroscopy (HETEROCOSY) spectra led to the assignment of all proton and carbon signals, which were consistent with the structure of aristolochene (**2**).†† This assignment was unambiguously confirmed by direct comparison with synthetic (±)-aristolochene.††† The absolute configuration of the *A. terreus* aristolochene is currently under investigation.

Although neither cadinene nor aristolochene have been previously reported as fungal metabolites, oxidized derivatives of each of these sesquiterpene hydrocarbons are known. Thus (−)-γ-cadinene has been suggested as the parent hydrocarbon of avocettin (**3**) (heptelidic acid), previously isolated from several fungal sources, including *Anthostoma avocetta*.[25,26] Aristolochene is a plausible precursor of a family of

† Authentic (+)-γ-cadinene, as well as γ-murolene, were kindly provided by Dr. YOKO NAYA of the Suntory Institute for Bioorganic Research, Osaka, Japan.

†† The ^{13}C NMR spectrum of aristolochene has previously been assigned.[31] The current assignments are in complete agreement with those reported earlier except that the signal attributed to the olefinic methine (C-9) was listed as 122.9 ppm. Professor STOTHERS has informed us that this number should be corrected to 118.65, in agreement with our own data on **2**. (Private communication, Professor J. B. STOTHERS, University of Western Ontario, London, Ontario, Canada).

††† Synthetic (±)-aristolochene was kindly provided by Professor EDWARD PIERS of the Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada. We also thank Professor ROBERT COATES of the Department of Chemistry, University of Illinois, Urbana, Illinois, U.S.A. for comparison samples of synthetic valencene and spectra of synthetic eromophilene.

fungal toxins isolated from *Penicillium roqueforti*, represented by eremofortin B (**4**),[27~29] and the *Aspergillus oryzae* metabolite sporogen-AO1 (**5**).[30] Further work on the terpenoid cyclases which mediate the formation of these hydrocarbons is in progress.

Acknowledgment

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