

Natural Aromatic Steroids as Potential Molecular Fossils from the Fruiting Bodies of the Ascomycete *Daldinia concentrica*

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The identification of aromatic steroid hydrocarbons bearing a methyl group at positions 1, 2, 3, 4, or 6 in sediments and petroleum has been puzzling since possible steroidal precursors have not yet been reported in living organisms. Two new aromatic steroids, (17 β ,20 R ,22 E ,24 R)-19-norergosta-1,3,5,7,9,14,22-heptaene (**1**) and (17 β ,20 R ,22 E ,24 R)-1-methyl-19-norergosta-1,3,5,7,9,14,22-heptaene (**2**), were isolated from the ascomycete *Daldinia concentrica*, of which compound **2** bears an unusual methyl group at position 1. We propose that the origin of these compounds is derived from the transformation undergone by their precursor due to microbial action. Compounds **1** and **2** could be the long-sought, biological precursor steroids for organic matter in Earth's subsurface.

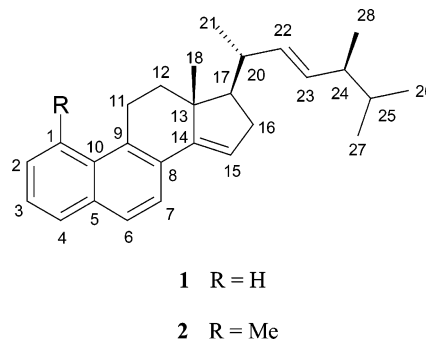
Biological marker compounds provide useful tools for evaluating the depositional environments of recent and ancient sediments and petroleum, largely through established relationships between the organic matter of sediments and source organisms.¹ The steroid skeleton occurs widely in sediments and crude oils; as a result of the information content inherent in compound structures and their often specific occurrence in organisms, steroids are extremely useful for geochemical purposes, in particular for correlating petroleum with their source rocks. They also yield interesting information on the origin of geological organic matter and its thermal maturation, as well as on paleoenvironmental conditions and paleoecological relationships.²

The 4-methyl steroid derivatives found in ancient sediments and crude oils have for a long time been the only steroids known to bear an extra methyl group on their skeleton.^{3,4} They are presumably molecular fossils of 4 α -methylsterols that occurred in microscopic algae, the dinoflagellates.¹ However, steranes with an additional methyl group at position 2 α or 3 β ,⁵ 3 β -carboxysteranes,² C₂₆–C₂₉ triaromatic steroid derivatives with a methyl at position 1 or 4,⁶ and C₂₁–C₂₉ triaromatic steroid hydrocarbons bearing a methyl group at position 2, 3, or 6 have been shown to occur in sediments of various geological ages.^{7,8} The identification of these compounds in sediments and petroleum is puzzling since possible steroidal precursors are rare in living organisms. Moreover, the occurrence of 3 β -carboxysteranes and 3-methyltriaromatic steroid hydrocarbons in various sediments is of particular importance because nearly all biological sterols bear a 3 β -hydroxyl group that relates to biochemical functions.

Aromatization is a commonly occurring process in the subsurface.^{9,10} It is interesting to note that human aromatase similarly catalyzes the formation of aromatic C₁₈ estrogenic steroids from C₁₉ androgens. This enzyme has received considerable attention because of the central importance of estrogen in many reproductive and metabolic processes. The synthesis of estrogen is required for the normal expression of secondary sexual characteristics and maintenance of pregnancy. Fetal expression in the brain

is believed to determine male or female metabolic patterns expressed during adult development.¹¹

Several hypotheses can be formulated in order to explain the origin and formation of these novel triaromatic steroid hydrocarbons in the subsurface. One point of information is that clay minerals play an important role as catalysts during their formation. Aromatization of the steroid skeleton may start either in ring A in immature samples or, after a backbone rearrangement, in ring C, depending on the conditions prevailing in the sediments. Further aromatization of monoaromatic steroids may then take place during the maturation of sediments and follow pathways A \rightarrow C or C \rightarrow A with rearrangement or loss of the C-19 methyl group.⁶ On the other hand, the relative concentrations of the 2-methyl and 3-methyl isomers increase with depth as compared with their 4-methyl counterparts. It is therefore likely that they could derive from the latter by a methyl shift, leading to the more stable 2-methyl and 3-methyl components. The implied rearrangement could be catalyzed by acid sites in the sediment matrix, in particular those occurring in clay minerals.⁷ However, the origin of the two 3 β -carboxysteranes isolated from a carbonated sediment is unknown. Among the various hypotheses which can be considered are as follows: (i) methylation at C-3 of the corresponding Δ^2 -sterene (Δ^2 -sterenes are commonly encountered in recent sediments)¹² followed by oxidation of the methyl group; (ii) addition of a carboxylic group to the corresponding Δ^2 -sterene, mediated by microorganisms.²



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In the present study, we report the characterization of two compounds, (17 β ,20 R ,22 E ,24 R)-19-norergosta-

1,3,5,7,9,14,22-heptaene (**1**) and (17 β ,20 R ,22 E ,24 R)-1-methyl-19-norergosta-1,3,5,7,9,14,22-heptaene (**2**), isolated from the ascomycete *Daldinia concentrica* (Bolton: Fries) Cesati & De Notaris (Xylariaceae). As far as we are aware, diaromatic and 1-methyl diaromatic steroid hydrocarbons have never been found from any living organism. Compounds **1** and **2** could be the long-sought, biological precursor steroids from living organisms for organic matter in the subsurface. Their existence provides a link between biological marker compounds or fossil molecules and biological origin. Moreover, these compounds potentially can be used as biological markers for the contribution of microorganism to sediments.

D. concentrica has afforded a great deal of scientific interest because of its unique secondary metabolites. Allport and Bu'Lock studied European and American *Daldinia* sp. in 1958 and 1960,^{13,14} which resulted in the identification of characteristic metabolites in their stromata and cultures. Some of those compounds were found to have antimicrobial and nematocidal activities.¹⁵ During more recent studies on *Daldinia* sp., more than 20 new metabolites have been discovered, including benzoquinones,¹⁶ a binaphthyl,¹⁷ cytochalasins,^{18–20} a daldiniapyrone,²¹ daldinones,²¹ heptentriols,²² triterpenoids,^{21,23,24} and derivatives of azaphilone²⁵ and benzophenone,¹⁷ of which some show a range of biological activities.

Compound **1** was isolated as pale yellow needles, $[\alpha]_D^{26} -51.42^\circ$. The molecular formula was determined to be C₂₇H₃₄ from the HRFABMS and ¹³C NMR data. Twenty-seven signals appeared in the ¹³C NMR spectrum (C \times 6, CH \times 13, CH₂ \times 3, CH₃ \times 5). The ¹H NMR spectrum showed the presence of a singlet methyl peak [δ 0.95 (3H, s, H-18)] and four doublet methyl peaks [δ 1.11 (3H, d, J = 6.5 Hz, H-21), 0.79 (3H, d, J = 6.9 Hz, H-26), 0.83 (3H, d, J = 6.9 Hz, H-27), 0.92 (3H, d, J = 6.8 Hz, H-28)]. The structure of the side-chain was determined from the EIMS and the ¹H–¹H COSY and HMBC data. A fragment ion was observed at m/z 232 due to the loss of a C₉H₁₈ peak from m/z 358 [M⁺] and thus suggested a monounsaturated C₉ side-chain. In the ¹H–¹H COSY spectrum, the correlations between H-17 and H-20; H-20 and H-21, 22; H-22 and H-23; H-23 and H-24; H-24 and H-25, 28; and H-25 and H-26, 27 were observed. Further correlations observed from H-17, H-21, and H-24 to C-22 and from H-20, H-25, and H-28 to C-23 in the HMBC spectrum confirmed the presence of a 24-methyl- Δ^{22} -sterol side-chain. The peak appearing at 968 cm^{–1} in the IR spectrum suggested a *trans* double bond at C-22. The cross-peak between H-11 and H-12 in the ¹H–¹H COSY spectrum and the correlations observed from H-18 to C-12, C-14, and C-17 in the HMBC spectrum indicated a methyl (CH₃-18) connected to C-13 and a double bond located at position C-14. By analysis of the HMQC, HMBC, and ¹H–¹H COSY NMR data, five other C=C bonds were located at positions 1, 3, 5, 7, and 9, respectively. The resonance of the C-28 methyl carbon at a characteristic value of δ_C 17.6 \pm 0.1 suggested a 24 R epimer.²⁶ In the NOESY spectrum, a cross-peak was observed between H-18 and H-20, so that the stereochemistry at C-17 and C-20 was assigned as β and R .²⁷ Thus, the structure of compound **1** was established as (17 β ,20 R ,22 E ,24 R)-19-norergosta-1,3,5,7,9,14,22-heptaene. This compound was synthesized previously.²⁸

Compound **2** was obtained as pale green needles. The EIMS gave a molecular ion peak at m/z 372. The elemental formula was determined to be C₂₈H₃₆, which was also proved by HRTOFMS ([M + H]⁺ 373.2885 calcd for C₂₈H₃₇ 373.2895). On comparing the IR and UV data with those

Table 1. ¹H and ¹³C NMR Spectral Data of **1** and **2** in CDCl₃

position	1		2	
	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	123.7 CH	7.97 d, 8.4	135.5 C	
2	126.0 CH	7.45 m	130.6 CH	7.22 m
3	125.2 CH	7.39 m	124.8 CH	7.22 m
4	128.4 CH	7.73 d, 8.0	127.5 CH	7.57 m
5	132.7 C		133.7 C	
6	126.3 CH	7.58 m	127.8 CH	7.57 m
7	123.6 CH	7.63 m	123.2 C	7.65 d, 3.7
8	128.4 C		129.2 C	
9	130.1 C		134.6 C	
10	132.7 C		132.4 C	
11	23.7 CH ₂	3.27 m; 3.10 m	28.7 CH ₂	3.53 m
12	36.5 CH ₂	2.32 m; 1.65 m	37.2 CH ₂	2.25 m; 1.57 m
13	45.2 C		44.2 C	
14	148.1 C		148.6 C	
15	120.7 CH	6.09 bs	120.8 CH	6.12 bs
16	36.9 CH ₂	2.36 m; 2.15 m	36.7 CH ₂	2.39 m; 2.15 m
17	57.1 CH	1.73 m	57.3 CH	1.73 m
18	15.4 CH ₃	0.95 s	14.9 CH ₃	1.06 s
20	39.0 CH	2.32 m	39.0 CH	2.35 m
21	21.7 CH ₃	1.11 d, 6.5	21.1 CH ₃	1.12 d, 6.6
22	135.3 CH	5.22 m	135.3 CH	5.25 m
23	132.4 CH	5.29 m	132.4 CH	5.32 m
24	42.9 CH	1.85 m	42.9 CH	1.89 m
25	33.1 CH	1.46 m	33.1 CH	1.49 m
26	20.0 CH ₃	0.79 d, 6.9	20.0 CH ₃	0.86 d, 6.3
27	19.7 CH ₃	0.83 d, 6.9	19.7 CH ₃	0.86 d, 6.3
28	17.7 CH ₃	0.92 d, 6.8	17.7 CH ₃	0.95 d, 6.8
Me			27.3 CH ₃	2.93 s

of compound **1**, compound **2** could be assigned the same basic skeleton as compound **1**. Six methyl signals (δ 14.9, 17.7, 19.7, 20.0, 21.1, and 27.3) appeared in the ¹³C NMR spectrum and indicated compound **2** to be a methyl-substituted 19-norergosta-1,3,5,7,9,14,22-heptaene. The location of this methyl was established by NOESY experiment since a strong NOE between the methyl group and H-11 was observed. ¹H–¹H COSY, HMQC, and HMBC NMR experiments confirmed the proposed structure of **2**. The stereochemistry at C-17, C-20, C-22, and C-24 was determined to be β , R , E , and R , respectively, on the basis of the same analysis as used for compound **1**. Thus, the structure of compound **2** was assigned as (17 β ,20 R ,22 E ,24 R)-1-methyl-19-norergosta-1,3,5,7,9,14,22-heptaene.

Experimental Section

General Experimental Procedures. Melting points obtained on an XRC-1 apparatus are uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were obtained with a UV-210 spectrometer. IR spectra were obtained with a Bio-Rad FTS-135 instrument. NMR spectra were recorded on a Bruker DRX-500 spectrometer with TMS as an internal standard. EIMS and HRFABMS were recorded with a VG Autospec-3000 spectrometer. HRTOFMS was recorded with a API QSTAR Pulsar 1 mass spectrometer. Silica gel (200–300 mesh) and precoated silica gel GF₂₅₄ plates (Qingdao Marine Chemical Factory, Qingdao, People's Republic of China) were used for column chromatography and TLC, respectively.

Fungal Material. Fruiting bodies of *Daldinia concentrica* were collected in Laojunshan, Yunnan, People's Republic of China, in July 2003 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (HKAS 40992) was deposited at the herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and Isolation. Dried and powdered fruiting bodies of *D. concentrica* (11.5 kg, dry weight) were extracted with CHCl₃ (50 L \times 4) at room temperature. The extract (340

g) was subjected to silica gel column chromatography (CHCl₃/MeOH, 100:0, 95:5, 9:1) to give 21 fractions. Fraction 1 (eluted with CHCl₃, 1 g) was separated by silica gel column chromatography (petroleum ether/acetone, 100:0, 98:2, 95:5, 9:1, 8:2) and produced 22 crude fractions (1a–1v). Fraction 1e (eluted with petroleum ether, 15 mg) was purified by preparative TLC (petroleum ether/acetone, 99:1; UV detection at 254 nm) to yield pale yellow needles (**1**, 3.8 mg). Fraction 1f (eluted with petroleum ether, 34 mg) was purified by preparative TLC (petroleum ether/acetone, 99:1; UV detection at 254 nm) to yield pale green needles (**2**, 11.7 mg).

(17 β ,20R,22E,24R)-19-Norergosta-1,3,5,7,9,14,22-heptene (1): pale yellow needles; mp 124.5–125.5 °C (petroleum ether/acetone); [α]_D²⁶ –51.42° (c 0.84 mg/mL, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 309 (4.18), 297 (4.25), 287 (4.20), 269 (4.56), 258 (4.63), 240 (4.52) nm; IR (KBr) ν_{\max} 2957, 2925, 2870, 1630, 1511, 1458, 1369, 968, 809, 775, and 746 cm^{–1}; NMR data, see Table 1; EIMS m/z 358 [M]⁺ (14), 259 (20), 246 (31), 232 (100), 217 (32); HRFABMS m/z [M]⁺ 358.2622 (calcd for C₂₇H₃₄, 358.2661).

(17 β ,20R,22E,24R)-1-Methyl-19-norergosta-1,3,5,7,9,14,22-heptaene (2): pale green needles; mp 123.5–124.5 °C (petroleum ether/acetone); [α]_D²⁶ –48.15° (c 2.25 mg/mL, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 351 (2.82), 333 (3.05), 314 (4.12), 301 (4.20), 292 (4.06), 273 (4.45), 263 (4.55), 257 (4.50) nm; IR (KBr) ν_{\max} 2956, 2925, 2877, 1634, 1560, 1453, 1368, 968, 831, 815, and 760 cm^{–1}; NMR data, see Table 1; EIMS m/z 372 [M]⁺ (29), 273 (22), 246 (100); HRTOFMS m/z [M + H]⁺ 373.2885 (calcd for C₂₈H₃₇, 373.2895).

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Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- Robinson, N.; Eglinton, G.; Brassell, S. C. *Nature* **1984**, *308*, 439–442.
- Dany, F.; Riolo, J.; Trendel, J. M.; Albrecht, P. *J. Chem. Soc., Chem. Commun.* **1990**, 1228–1230.
- Rubinstein, I.; Albrecht, P. *J. Chem. Soc., Chem. Commun.* **1975**, 957–958.
- Ensminger, A.; Joly, G.; Albrecht, P. *Tetrahedron Lett.* **1978**, *19*, 1575–1578.
- Summons, R. E.; Capon, R. J. *Geochim. Cosmochim. Acta* **1977**, *52*, 2733–2736.
- Ludwig, B.; Hussler, G.; Wehrung, P.; Albrecht, P. *Tetrahedron Lett.* **1981**, *22*, 3313–3316.
- Lichtfouse, E.; Riolo, J.; Albrecht, P. *Tetrahedron Lett.* **1990**, *31*, 3937–3940.
- Lichtfouse, E.; Albrecht, P. *Tetrahedron* **1994**, *50*, 1731–1744.
- Blumer, M. *Sci. Am.* **1976**, *234*, 34–35.
- Albrecht, P.; Ourisson, G. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 209–225.
- Zhou, D.; Korzekwa, K. R.; Poulos, T.; Chen, S. *J. Biol. Chem.* **1992**, *267*, 762–768.
- Dastillung, M.; Albrecht, P. *Nature* **1977**, *269*, 678–679.
- Allport, D. C.; Bu'lock, J. D. *J. Chem. Soc.* **1960**, 654–662.
- Allport, D. C.; Bu'lock, J. D. *J. Chem. Soc.* **1958**, 4090–4094.
- Anke, H.; Stadler, M.; Mayer, A.; Sterner, O. *Can. J. Bot.* **1995**, *73*, 802–810.
- Qin, X. D.; Liu, J. K. *Helv. Chim. Acta* **2004**, *87*, 2022–2024.
- Hashimoto, T.; Tahara, S.; Takaoka, S.; Tori, M. *Chem. Pharm. Bull.* **1994**, *42*, 1528–1530.
- Buchanan, M. S.; Hashimoto, T.; Takaoka, S.; Kan, Y.; Asakawa, Y. *Phytochemistry* **1996**, *42*, 173–176.
- Buchanan, M. S.; Hashimoto, T.; Asakawa, Y. *Phytochemistry* **1996**, *41*, 821–828.
- Buchanan, M. S.; Hashimoto, T.; Asakawa, Y. *Phytochemistry* **1995**, *40*, 135–140.
- Quang, D. N.; Hashimoto, T.; Tanaka, M.; Baumgartner, M.; Stadler, M.; Asakawa, Y. *J. Nat. Prod.* **2002**, *65*, 1869–1874.
- Wang, F.; Liu, J. K. *Helv. Chim. Acta* **2004**, *87*, 2131–2134.
- Quang, D. N.; Hashimoto, T.; Tanaka, M.; Baumgartner, M.; Stadler, M.; Asakawa, Y. *Phytochemistry* **2002**, *61*, 345–353.
- Stadler, M.; Baumgartner, M.; Grothe, T.; Muehlbauer, A.; Seip, S.; Wollweber, H. *Phytochemistry* **2001**, *56*, 787–793.
- Hashimoto, T.; Tahara, S.; Takaoka, S.; Tori, M. *Chem. Pharm. Bull.* **1994**, *42*, 2397–2399.
- Wright, J. L. C.; McInnes, A. G.; Shimizu, S.; Smith, D. G.; Walter, J. A. *Can. J. Chem.* **1978**, *56*, 1898–1903.
- Yaoita, Y.; Amemiya, K.; Ohnuma, H.; Furumura, K.; Masaki, A.; Matsuki, T.; Kikuchi, M. *Chem. Pharm. Bull.* **1998**, *46*, 944–950.
- Dannenberg, H.; Hebenbrock, K. F. *Justus Liebigs Ann. Chem.* **1966**, *700*, 106–119.

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