Tetrahedron Vol. 42, No. 16, pp. 4475 to 4480, 1986
Printed in Great Britain.

0040-4020/86 $3.00+.00
Pergamon Journals Ltd.

NEW TRANS-FUSED AFRICANOLS FROM LEPTOGRAPHIUM LUNDBERGII

WOLF-RAINER ABRAHAM*, LUDGER ERNST, LUDGER WITTE
GBF - Gesellschaft für Biotechnologische Forschung mbH
Mascheroder Weg 1, D-3300 Braunschweig, FRG

HANS-PETER HANSSEN, EWALD SPRECHER
Institut für Angewandte Botanik, Abt. Pharmakognosie
Bundesstr. 43, D-2000 Hamburg, FRG

(*Received in Germany* 21 *April* 1986)

Abstract

Three new sesquiterpene alcohols with africanane skeleton - leptographiol (1), isoleptographiol (2), and isoafricanol (3) - have been isolated from Leptographium lundbergii Lag. et Mel.. Their structures and configurations were elucidated by spectroscopic methods, in particular by nuclear Overhauser enhancement difference ^1H NMR spectra and by two-dimensional $^1H/^1H$- and $^{13}C/^1H$-chemical shift correlation. The biosynthetic origin of 1 - 3 is briefly discussed.

Introduction

Leptographium lundbergii Lag. et Melin is a sapwood staining ascomycete fungus causing a dark, blue discolouration of the infected wood. The fungus is mainly found on pine logs and lumbers in North and West Europe. Spores are distributed by insects or air. The taxonomic disposition of Leptographium is not generally agreed upon [1]; a differentiation from the related genus Ceratocystis is accomplished by a morphologically distinct imperfect stage. Chemosystematic investigations have shown that many species of these two ascomycete genera produce volatile terpenes [2 - 4]. From the steam distillates of the newly investigated species, L. lundbergii, grown on a synthetic liquid culture medium, three new tricyclic sesquiterpene alcohols have been isolated which are described here for the first time as natural products.

Results

Leptographium lundbergii Ha 2/82 was isolated from timber logs in Friedrichsruh near Hamburg/FRG. As major components, three compounds of the composition $C_{15}H_{26}O$ were isolated from its cultures. Their IR spectra revealed an absorption of a hydroxyl group and their ^{13}C NMR spectra showed no resonances of sp^2-carbons, thus indicating tricyclic sesquiterpene alcohols. Each of the three compounds showed three ^1H NMR resonances between 0.66 and 0.12, so a triply substituted cyclopropane moiety was likely to be present in all of them. These results were the first hint of three isomeric alcohols all having similar carbon skeletons.

The results of $^1H(^1H)$ decoupling experiments on the main compound 1 and the signal multiplicities in the ^{13}C NMR spectrum led to the africanane skeleton [5]. Starting from the cyclopropyl proton signals, the protons at C-5, C-7, and C-8 could be assigned via homonuclear double resonance or 2D chemical shift correlation ("COSY"). 5β-H showed a long range coupling to 7β-H of 2.3 Hz, requiring the intervening dihedral angles to be both close to 180°. This points to a trans-fused cyclopentane ring because the cis-configuration does not permit such an arrangement.

The characteristic chemical shifts and the SFORD multiplicities allowed the assignments of C-2, C-4, C-6, and C-9 whereas C-3, C-5 and C-7 could be identified through 2D $^{13}C/^1H$ shift correlation via $^1J_{CH}$ using known proton assignments. $^{13}C/^1H$ shift correlation via $^nJ_{CH}$ (n = 2 or 3), optimized for couplings of 6 Hz, revealed the remaining assignments. Thus, the methyl singlet at δ_H = 0.962 gave cross-peaks with the C-2 and C-3 resonances and had therefore to be attributed to 12-H. The methyl singlet at δ_H = 1.250 correlated with the peaks at δ_C = 48.15 (C-8) and 41.55 (C-10), so it

was identified as the carbinyl methyl (CH_3-15). This information simultaneously permitted the distinctions of C-1 from C-8 and of C-10 from C-11 as indicated. The remaining methyl singlets gave correlations with C-5, C-6, and C-7 and belong to the geminal methyl groups at C-6.

The following evidence is in agreement only with a trans-fused cyclopentane ring:

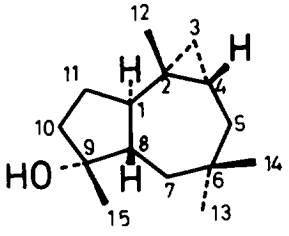
i) The large ^4J coupling between 5β-H and 7β-H (W arrangement);

ii) the distinct broadening of only one methyl proton singlet at $\delta = 0.987$ because of ^4J couplings of the axial methyl group (14-H) with two antiperiplanar protons, 5α-H and 7α-H. In the cis-configuration each of the geminal methyl groups should experience one ^4J coupling, giving similar line-widths for the two resonances;

iii) the large ^{13}C shift difference between the geminal methyl groups: $\delta_{C\text{-}13} = 34.06$, $\delta_{C\text{-}14} = 24.47$, $\Delta\delta_C = 9.59$ resulting from the gauche-interactions of C-14 with C-8 and C-4 while C-13 has none. In the cis-situation one would expect similar chemical shifts for the methyl carbons because each methyl group would experience one gauche interaction;

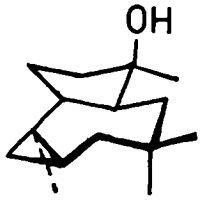
iv) the ^3J couplings between 8-H and the two protons at C-7 (11.8 and 2.3 Hz). Dreiding models show the appropriate torsional angles to be ca. 165° and ca. 75° in the trans-configuration, from which coupling constants of 12.3 and 2.4 Hz are calculated by means of the "empirical Karplus-Conroy equation" [6], in good agreement with the experimental findings. For the cis-configuration torsional angles of ca. 145° and ca. 100° are measured which lead to calculated values of 9.5 and 2.5 Hz, the first one being distinctly worse than in the trans case.

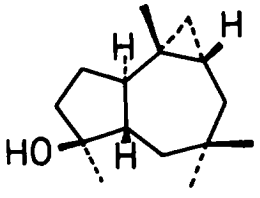
Once the methyl signals had been assigned, the configuration of C-9 could be determined by NOE experiments. Irradiation of the 15-H resonance gave a NOE at 7β-H (and effects in the multiplet of the cyclopentane protons). This points to the β-orientation of the methyl substituent. An α-methyl group should give a larger NOE at 7α-H than at 7β-H, yet no effect at 7α-H was observed. Irradiations of the remaining methyl signals confirmed that 1 is (1R*,2R*,4S*,8S*,9R*)-2,6,6,9-tetramethyltricyclo[6.3.0.$0^{2,4}$]undecan-9-ol. The NMR data for this and the following compounds are given in Tables 1 (chemical shifts and coupling constants) and 2 (nuclear Overhauser effects).



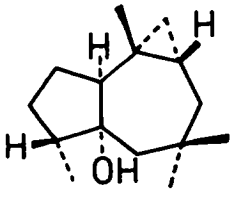
1

≡

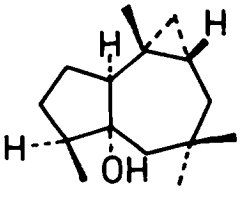




2



3



4

Compound 2, of very similar properties as 1, was also isolated, however in much smaller amounts. In the ^1H NMR spectrum the coupling pattern of the 3- and 7-membered rings are identical to 1, with coupling constants differing by less than 0.7 Hz. The ^{13}C NMR spectra of 1 and 2 are also very similar (Table 1). Deviations of more than 0.5 ppm in the chemical shifts are only found for C-7

TABLE 1: NMR chemical shifts and coupling constants of 1 - 4 (in $CDCl_3$)

^1H NMR (400.1 MHz)

	1	2	3
1α-H	m 1.61	ddd 1.31	m 1.74
3α-H	dd 0.213	dd 0.17	dd 0.121
3ß-H	dd 0.525	dd 0.53	dd 0.388
4ß-H	dddd 0.454	dddd 0.47	dddd 0.663
5α-H	dd 1.092	dd 1.03	dd 1.264
5ß-H	ddd 1.795	ddd 1.80	ddd 1.670
7α-H	dd 1.097	dd 0.92	dd 1.462
7ß-H	ddd 1.437	ddd 1.52	d 1.534
8ß-H	m 1.65	ddd 1.87	-
9ß-H	-	-	ddq 1.564
10α-H	m 1.85	m 1.61±0.04	m 1.37
10ß-H	to	m 1.69±0.02	m 1.7
11α-H		m 1.53±0.01	m 1.84
11ß-H	1.50	m 1.73±0.02	m 1.7
12-H	s 0.962	s 1.028	s 0.904
13-H	s 0.918	s 0.903	s 1.094
14-H	s 0.987	s 0.994	s 0.960
15-H	s 1.250	s 1.078	d 0.888

J(Hz): 1: 1α,8ß-8-10; 3α,3ß-4.3; 3α,4ß-4.8; 3ß,4ß-8.3; 4ß,5α-10.9; 4ß,5ß-6.3; 5α,5ß-14.5; 5ß,7ß-2.3; 7α,7ß-12.8; 7α,8ß-11.8; 7ß,8ß-2.3

2: 1α,8ß-10.3; 1α,11α-9.5; 1α,11ß-5.1; 3α,3ß-3.9; 3α,4ß-4.7; 3α,12>0; 3ß,4ß-8.3; 4ß,5α-10.5; 4ß,5ß-6.2; 5α,5ß-14.5; 5ß,7ß-2.5; 7α,7ß-12.9; 7α,8ß-12.4; 7ß,8ß-2.7; 10ß,15>0

3: 3α,3ß-4.1; 3α,4ß-5.1; 3ß,4ß-8.3; 4ß,5α-12.3; 4ß,5ß-4.2; 5α,5ß-14.1; 5ß,7α-1.1; 7α,7ß-14.8; 9ß,15-6.7; 9ß,10α-11.9; 9ß,10ß-6.8; unresolved long-range couplings (from COSY) between 12-H and 3α-, 3ß-, 4ß-, 5ß-H; 13-H and 5ß-, 7ß-, 14-H; 14-H and 5α-, 7α-, 13-H; 15-H and 1α-H

^{13}C NMR (75.5 MHz)

	1	2	3	4[5,e]
C-1	48.73 d	48.87 +[a,b]	54.06 +[a]	56.2 d
C-2	20.60 s	20.75 0	18.45 0	18.5 s
C-3	23.76 t	23.70 -[c]	22.76 -[b]	23.9 t
C-4	21.90 d	21.90 +	21.02 +	20.9 t
C-5	43.40 t	43.67 -[d]	41.06 -	40.6 t
C-6	33.30 s	33.46 0	34.10 0	34.5 s
C-7	43.36 t	45.08 -[d]	46.51 -	45.3 t
C-8	48.15 d	48.74 +[b]	85.81 0	87.3 s
C-9	81.27 s	80.34 0	45.74 +	50.3 d
C-10	41.55 t	42.07 -[d]	31.39 -	32.3 t
C-11	23.35 t	23.07 -[c]	23.56 -[b]	26.2 t
C-12	19.62 q	19.80 +	22.14 +	23.2 q
C-13	34.06 q	34.07 +	31.16 +	31.7 q
C-14	24.47 q	24.17 +	31.67 +	31.9 q
C-15	25.73 q	22.47 +	12.33 +	15.6 q

[a]amplitude of signals in DEPT-135 spectrum (CH_3 or CH = +; CH_2 = -; quat. C = 0); [b,c,d]assignments may be interchanged; [e]assigned by comparison with 3.

to C-10 and, in particular, for C-15 (3.26 ppm). It is therefore resonable to assume that 2 is the 9-epimer of 1. This assumption is further justified by the results of some NOE experiments (Table 2). Notably, saturation of 15-H caused enhancements of the 1α-H, 7α-H and 10α-H resonances which can only be explained by the α-orientation of the methyl group at C-9 (cf. with 1 above). Hence 2 is (1R*,2R*,4S*,8S*,9S*)-2,6,6,9-tetramethyltricyclo[6.3.0.$0^{2,4}$]undecan-9-ol.

TABLE 2: Results of NOE experiments on 1 - 3

Compound	Resonance irradiated	Resonances enhanced
1	12	3β, 4β, 11β(?) (δ= 1.77 - 1.57)
	13	5α, 5β, 7α, 7β
	14[a]	3β, 4β, 5β, 7β, ? (δ= 1.71 - 1.58)
	15	7β, 10β(?) (δ= 1.71 - 1.57)
2	12 and 5α	1α, 3β, 4β, 5β, 8β, 11β
	13	5β, 7β, 14
	14	4β, 5β, 7β, 8β, 13
	15	1α, 7α, 10α, 11α
3	3α	1α, 3β, 5α
	12 and 15	3β, 4β, 7α, 7β, 9β, 11β
	13	5α, 5β, 7α, 14

[a] Due to insufficient selectivity, 12-H was also partially saturated.

Compound 3, also isolated only in small amounts, displayed three methyl singlets and one methyl doublet in its ^{1}H NMR spectrum. The ^{13}C NMR spectrum was similar [5] to that of africanol 4. The COSY spectrum revealed a number of long-range couplings to the methyl protons (Table 1) which were not resolved in the one-dimensional spectrum. This greatly facilitated the assignments. The ^{13}C NMR spectrum was then assigned by 2D-^{13}C/^{1}H chemical shift correlation. The stereochemistry of 3 was derived from NOE experiments (Table 2). These showed that 3 has the structure of 9-epi-africanol, in which the hydroxy group at C-8 and the methyl substituent at C-9 are cis to each other. This cis-arrangement is also reflected in the increased shielding of C-8, C-9, and C-15 in 3 relative to 4 (Table 1).

It is thought that the africanae skeleton originates from humulene [7], but our attempts to detect humulene in the distillate failed.

To our knowledge, africanane-type sesquiterpenes were hitherto found only in soft corals [5], Compositae [8], and Verbenaceae [9]; so the detection of leptographiol (1), isoleptographiol (2), and isoafricanol (3) is the first finding of this unusual skeleton in fungi.

Experimental

Leptographium lundbergii Ha 2/82 was isolated from timber logs in Friedrichsruh near Hamburg, FRG. The strain was cultivated on a defined synthetic liquid culture medium containing glucose (2%), asparagine (0.1%), and mineral salts [10] in 250ml-Erlenmeyer flasks. After 20 days, the cultures were harvested, and the volatiles were obtained by circulation steam distillation [11] or by extraction of the culture broth with dichloromethane. The crude extract was chromatographed on a Si-60 column, 63-125 μm (Merck) with n-hexane/ethyl acetate 97:3. From 1 liter of medium 34mg of leptographiol (1), 4mg of isoleptographiol (2), and 3mg of isoafricanol (3) were isolated.

EI mass spectra were recorded with an AEI MS 902S mass spectrometer at a resolution of 1000. High resolution data were obtained by the peak matching method at a resolution of 30.000. GC-MS experiments were performed at a AEI MS 30 mass spectrometer using a DB1-15N column (J&W

Scientific), oven temperature gradient from 70° to 300° with a rate of 6°C/min.

The ^{1}H NMR spectra were obtained at 400 MHz on a Bruker WM 400 spectrometer and the ^{13}C NMR spectra at 75.5 MHz on a Bruker AM 300 spectrometer. Each spectrometer is interfaced to an ASPECT 3000 computer equipped with an array processor and using Bruker standard DISNMRP software. The following solutions were used: a) 28 mg of 1 in 0.55 ml of $CDCl_3$ in a thin-walled 5 mm o. d. sample tube, b) ca. 4 mg of 2 in 0.2 ml of $CDCl_3$ in a thick-walled 5 mm o. d. sample tube, c) ca. 3 mg of 3 as under b). Tetramethylsilane was used as the internal reference for ^{1}H and the solvent signal (δ= 77.05) for ^{13}C.

In the proton NOE difference experiments [12] separate data sets were obtained for the on-resonance and the control spectra. The irradiation power was 40-50 dB below a nominal 0.2 W and the saturation delay 10 s. Two-dimensional ^{1}H/^{1}H shift correlation experiments ("COSY-90", quadrature detection by phase cycling in the F_1 dimension) [13] were run with relatively good digital resolution (0.7 - 1.4 Hz/point) to allow the recognition of multiplet patterns in cross-sections through the data matrices. This required the use of 512 increments in t_1. Accumulating 48 - 160 scans with relaxation delays of 1.0 s gave measuring times of 13 - 24 h. A sinebell window function was applied in both F_1 and F_2. Zero-filling in one or both dimensions resulted in data matrices of 1 or 2 megawords.

^{13}C NMR spectra were run both in the broadband decoupling mode (ca. 20 000 scans for 2 and 3) and in the single-frequency off-resonance decoupling mode (1) or using the DEPT [14] technique (2 and 3) with the flip angle of the final decoupler pulse equal to 135°. This gives spectra devoid of signals for quaternary carbons and distinguishes between methyl and methine signals on the one hand and methylene signals on the other. The relaxation delay was 1.0 s and the $(2J)^{-1}$ delay 3.45 ms.

The two-dimensional ^{13}C/^{1}H chemical shift correlation experiments (via $^1J_{CH}$) were carried out using the pulse sequence by Rutar [15] which suppresses vicinal and longer-range homonuclear couplings in the F_1 dimension, thereby increasing the signal/noise ratio (S/N) and permitting the investigation of smaller sample quantities than with the standard pulse sequence. Digital resolutions were 2.3 - 3.4 Hz/point in both dimensions. 2K Data points were sampled for 128 increments of t_1. 320 Scans were accumulated for 1 with a relaxation delay of 0.8 s and 1440 and 1600 scans (relaxation delay 0.5 s) for 2 and 3, respectively. Total measuring times were 15 h (1), 49 h (2) and 52 h (3). Zero-filling in t_1 to 256 points was applied giving 256K data matrices after 2D Fourier transformation. Sinebell multiplication in both dimensions and magnitude representation was used for the data of 1, but sinebell squared multiplication and power representation for 2 and 3 because of the low S/N. In the cases of 2 and 3 this low S/N allowed the observation of only the ^{13}C/^{1}H cross-peaks for the four methyl groups and for C/H-4, while for 1 the complete correlations were obtained. A ^{13}C/^{1}H shift correlation via $^{2,3}J_{CH}$ was also carried out for 1. The parameters were: polarization transfer and refocussing delays optimized for J_{CH} = 6 Hz, relaxation delay 0.5 S, 480 scans, experimental time 22.5 h, sinebell squared multiplication, power spectrum representation, other parameters as for the $^1J_{CH}$-correlation. In this experiment cross-peaks were only observed between methyl proton resonances and the signals of geminal or vicinal carbon atoms.

Leptographiol (1)

Viscous oil, IR ($CHCl_3$): 3620 cm^{-1}. - ^{1}H and ^{13}C NMR see Tables 1 and 2. - MS (m/e): 222.19770 (222.19835 calculated for $C_{15}H_{26}O$)(M^+)(18%), 207 (19%), 164 (36%), 95 (58%), 43 (100%).

$$\alpha^{23} = \frac{\begin{matrix}589\text{nm} & 578\text{nm} & 546\text{nm} & 436\text{nm} & 365\text{nm}\end{matrix}}{\begin{matrix}+17.6^\circ & +18.6^\circ & +21.1^\circ & +36.9^\circ & +59.6^\circ\end{matrix}} \quad (c = 1.0,\ CHCl_3)$$

Isoleptographiol (2)

Colourless oil, IR ($CHCl_3$): 3610 cm^{-1}. - ^{1}H and ^{13}C NMR data see Tables 1 and 2. - MS (m/e): 222.19792 (222.19835 calculated for $C_{15}H_{26}O$)(M^+)(25%), 207 (22%), 164 (40%), 95 (55%), 43 (100%).

$$\alpha^{23} = \frac{\begin{array}{cccc} 589nm & 578nm & 546nm & 436nm \\ +14.6^{\circ} & +16.1^{\circ} & +18.8^{\circ} & +30.7^{\circ} \end{array}}{} \quad (c = 0.63, CHCl_3)$$

Isoafricanol (3)

Colourless oil. IR ($CHCl_3$): 3610 cm^{-1}. - ^1H and ^{13}C NMR data see Tables 1 and 2. - MS (m/e): 222.19792 (222.19835 calculated for $C_{15}H_{26}O$)(M^+)(20%), 207 (12%), 204 (16%), 55 (98%), 43 (100%).

$$\alpha^{23} = \frac{\begin{array}{cccc} 589nm & 578nm & 546nm & 436nm \\ +11.4^{\circ} & +11.9^{\circ} & +13.3^{\circ} & +22.3^{\circ} \end{array}}{} \quad (c = 0.59, CHCl_3)$$

Acknowledgements

We are indebted to Prof. A. Käärik, Uppsala, Sweden, for the exact determination of the newly isolated strain. This work was in part (H. and S.) supported by the Deutsche Forschungsgemeinschaft.

References

1 Hunt, J. Lloydia 1956, 19, 1.
2 Sprecher, E.; Hanssen, H.-P. Antonie van Leeuwenhoek 1983, 49, 493-499.
3 Sprecher, E.; Kubeczka, K.-H.; Ratschko, M. Arch. Pharm. 1975, 308, 843-851.
4 Hanssen, H.-P. in: Essential Oils and Aromatic Plants (Baerheim Svendsen, A.; Scheffer, J. J. C. eds.), Dordrecht (NL), M. Nijhoff/Dr. W. Junk Publ., p. 173-177,(1985).
5 Tursch, B.; Braekman, J. C.; Daloze, D.; Fritz, P.; Kelecon, A.; Karlsson, R.; Losman, D. Tetrahedron Letters 1974, 747-750.
6 Günther, H. "NMR Spectroscopy", p. 106, Wiley, Chichester (1980).
7 Shirahama, H.; Hayana, K.; Kanemoto, Y.; Misumi, S.; Ohtsuka, T.; Hashiba, N.; Furusaki, A.; murata, S.; Noyari, R.; Matsumoto, T. Tetrahedron Letters 1980, 21, 4835-4838.
8 Bohlmann, F.; Zdero, C. Phytochemistry 1978, 17, 1669-1671.
9 Dartayet, G. H.; Catâlan, C. A.; Retamar, J. A.; Gros E. G. Phytochemistry 1984, 23, 688-689.
10 Sprecher, E. Planta 1959, 53, 565-574.
11 Sprecher, E. Dtsch. Apoth. Ztg. 1963, 103, 213.
12 Hall, L. D.; Sanders, J. K. M. J. Am. Chem. Soc. 1980, 102, 5703-5711; J. Org. Chem. 1981, 46, 1132-1138.
13 Bax, A.; Freeman, R.; Morris, G. J. Magn. Reson. 1981, 42, 164-168.
Bax, A.; Freeman, R. J. Magn. Reson. 1981, 44, 542-561.
14 Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. J. Magn. Reson. 1982, 48, 323-327.
15 Rutar, V. J. Magn. Reson. 1984, 58, 306-310.