NARDIIN, A NEW *ent*-KAURANE DITERPENOID FROM THE LIVERWORT *Nardia Scalaris**

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An *ent*-kaurane diterpenoid nardiin(II) was isolated from the chloroform extract of *Nardia scalaris* S. GRAY corr. TREV. Its structure was determined by means of ^{1}H NMR spectroscopy and garryfoline–cuauchichicine rearrangement as *ent*-15α-hydroxy-9(11),16-kauradien-6-one.

According to most botanists the liverworts represent an independent evolutionary line of plants and they are classified, together with mosses (*Musci*), among *Bryophyta* (*cf.* Hébant[1]). From the chemical point of view it is, therefore, characteristic of liverworts that they contain various terpenoid substances, primarily mono-, sesqui-, and diterpenoids, of which some are enantiomeric in comparison with similar compounds in higher plants[2].

In the case of the chemically little investigated liverwort *Nardia scalaris* S. GRAY corr. TREV. gas chromatographic analysis of the essential oil[3] was carried out and the same method detected lunularic acid with lunularin[4] and sorbitol[5]. Our group recently described[6] the presence of (+)-21α-methoxyserrat-14-en-3-one (*I*). The aim of this study is the description of nardiin, a new *ent*-kaurene diterpenoid, isolated now from this liverwort.

From the chloroform extract of dry liverwort we isolated compound *II*, m.p. 177–180°C by means of column chromatography and preparative HPLC. Its elemental composition was $C_{20}H_{28}O_2$ and its infrared spectrum displayed bands characteristic of the hydroxyl group (3 460 cm^{-1}), a keto group (1 689 cm^{-1}) and a double bond (1 659 cm^{-1}). The ^{1}H NMR spectrum demonstrated the presence of three tertiary methyl groups (singlets at δ 1·10, 1·14, and 1·15), an exomethylene group (unresolved multiplets at δ 5·12 and 5·15), a trisubstituted double bond in the vicinity of a methylene group (broadened triplet at δ 5·56 with J = 3·5 Hz, interacting with the multiplets at δ 2·09 and 2·52, belonging to the CH_2 group) and one CH—O hydrogen (multiplet at δ 4·14). *In situ* reaction with trichloroacetyl isocyanate demonstrated the presence of a secondary hydroxyl (CH—O hydrogen is shifted

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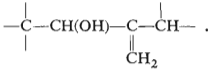
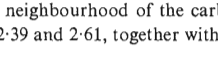
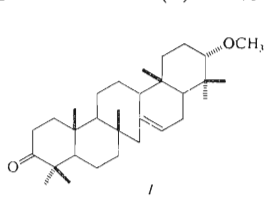
by acylation by 1·41 ppm downfield and the signal of the NH proton appears at δ 8·43). Only allylic couplings of the CH—O proton with the exomethylene hydrogens (^4J = 2·0 and 2·5 Hz) lead to its localization into the vicinity of the exomethylene group, the hydrogen atoms of which further interact merely with the proton at δ 2·78 which leads summarily to the formation of the fragment



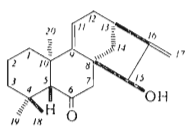
Further the hydrogens in the neighbourhood of the carbonyl group are also shifted downfield. The doublets at δ 2·39 and 2·61, together with the singlet at δ 3·11 indicate the fragment



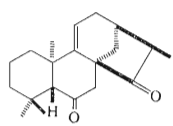
From these facts and from the optical rotation value ($[\alpha]_D = -29°$) it may be judged that the isolated diterpenoid has probably an *ent*-kaurane skeleton with a keto group in the position 6, a hydroxyl group in the position 15 and a double bond between the carbons $C_{(9)}$ and $C_{(11)}$. From the distinct upfield shift (−0·77 ppm) of the signlet $C_{(5)}$—H after acylation with trichloroacetyl isocyanate, in consequence of the change of the van der Waals effect of the sterically close $C_{(15)}$—OR group, the β-configuration of the hydroxyl group in the position 15 follows. This enabled a direct proof of the *ent*-kaurane skeleton by means of the garryfoline–cuauchichicine rearrangement[7]. Hydrogenation of nardiin (*II*) on 10% Pd—C (ref.[8]) afforded *III* in



I



II



III

TABLE I

Proton NMR parameters of compounds *II* and *III* in deuteriochloroform

Compound	Chemical shifts[a]														
	H-5	H-7	H-7′	H-11	H-12	H-12′	H-13	H-14	H-14′	H-15	H-17	H-17′	18-CH_3	19-CH_3	20-CH_3
II	3·11 s	2·39 bd	2·61 d	5·56 bt	2·09 dm	2·52 ddd	2·78 bd	1·54 d	1·70 dd	4·14 um	5·12 um	5·15 um	1·10[b] s	1·14[b] s	1·15[b] s
II[c]	2·34 s	2·55 bd	2·61 d	5·61 bt	2·24 dm	2·59 ddd	2·86 bt	1·64 d	1·91 dd	5·55 um	5·18 um	5·04 um	1·04[b] s	1·11[b] s	1·14[b] s
III[d]	2·67 s	2·36 d	2·47 d	5·59 bt	2·25 bdd	2·41 dt	[e]	[e]	1·97 bd	—	—	—	1·09[b] s	1·16[b] s	1·17[b] s

	Coupling constants											
	$J_{7,7'}$	$J_{11,12}$	$J_{11,12'}$	$J_{12,12'}$	$J_{12,13}$	$J_{12',13}$	$J_{13,14}$	$J_{14,14'}$	$J_{15,OH}$	$J_{16,17}$	$J_{17,17'}$	$J_{13,14'}$
II[f]	19·5	3·5	3·5	17·5	2·5	4·5	0	11·5	10·5	—	[e]	5·0
III	19·5	4·0	2·5	18·0	2·5	4·0	[e]	[e]	—	6·4	—	[e]

[a] Signals of protons in positions 1, 2 and 3 could not be assigned; [b] The assignment of the methyl signals can be interchanged; [c] After the addition of trichloroacetyl isocyanate. The NH proton appears as a singlet at δ 8·43; [d] 17-Methyl group gives a doublet at δ 1·16 with $J = 6{\cdot}4$ Hz; [e] Chemical shifts or coupling constants were not determined; [f] $J_{15,17} = 2{\cdot}0$ and $J_{15,17'} = 2{\cdot}5$ Hz.

quantitative yield, $C_{20}H_{28}O_2$, m.p. 136–137°C. In the infrared spectrum the band of the hydroxyl group disappeared and a peak indicating the presence of a five-membered cyclic ketone (1 737 cm^{-1}) appeared. In the ^1H NMR spectrum the signals of the exomethylene hydrogens and the CH—O hydrogen disappeared and the signal of the secondary methyl (doublet at δ 1·16 with J = 6·4 Hz) appeared (Table I). These facts show that the structure of the investigated compound is (16*R*)-*ent*-9(11)--kaurene-6,15-dione (*III*). The garryfoline–cuauchichicine rearrangement demonstrated unambiguously the *ent*-kaurane skeleton as well as the position and the configuration of the hydroxyl group in compound *II*. This also permits the assignment of the double bond in *II* and *III* into the position between the carbons 9 and 11 and of the carbonyl group into position 6, so that nardiin (*II*) must have the structure of *ent*-15α--hydroxy-9(11),16-kauradien-6-one.

EXPERIMENTAL

Column chromatography was carried out on silica gel according to Pitra[9], particle size 60–90 μm, with the addition of 10% of water. Preparative HPLC was carried out on Silpearl for TLC (Kavalier, Votice, Czechoslovakia), in a glass column (25 × 250 mm) using gradient elution with n-hexane–ethyl acetate, at a 10 ml/min flow rate. A home-made instrument was used and the volume of fractions was 5 ml. Detection of the peak in the fractions was done by TLC. The melting points were measured on a Kofler block, the ^1H NMR spectra on a Varian XL-200 instrument (200 MHz) in the FT mode, the IR specta on a Perkin–Elmer 580 spectrophotometer, the mass spectra on an AEI-902 instrument and the CD spectra on a Roussel–Jouan CD-185 spectrograph.

Isolation of *II*

The chloroform extract (39·16 g) of dried *N. scalaris* (4 190 g), collected in June 1976 in Smědava (Jizera mountains), was chromatographed on silica gel with benzene with 1% of ether and then on the HPLC column. Fractions eluted with 4% of ethyl acetate in hexane (50 mg) afforded after crystallization from n-hexane a substance with m.p. 177–180°C, with the composition $C_{20}H_{28}O_2$. Mass spectrum: 300 ($C_{20}H_{28}O_2$, high resolution), 285 ($C_{19}H_{25}O_2$), 282, 257, 243, 239, 215. IR spectrum, ν_{max}^{KBr} cm^{-1}: 3 460 (OH), 1 689 (C=O), 1 659 (C=C). CD spectrum, (*c* 0·11, dioxane): $\Delta\varepsilon_{300}$ −2·76, $\Delta\varepsilon_{241}$ 0, $\Delta\varepsilon_{215}$ +14·33. $[\alpha]_D^{22}$ −29° (*c* 1·2, chloroform). ^1H NMR spectrum see Table I.

Rearrangement of *II*

Compound *II* (10 mg) in ethyl acetate (6 ml) was stirred for 30 min under hydrogen in the presence of 10% Pd–C (10 mg). After filtration and evaporation of the solvent diketone *III* was obtained, m.p. 136–137°C. IR spectrum, $\nu_{max}^{CCl_4}$ cm^{-1}: 1 711 (six-membered cyclic C=O), 1 737 (five-membered cyclic C=O), 1 403 (—CH_2—CO), 1 632 and 3 040 (C=C—H). ^1H NMR spectrum see Table I. Composition $C_{20}H_{28}O_2$ according to high-resolution mass spectrometry.

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