Dasypogalactone, a New C_3-Symmetric Macrolactone from the Indonesian Lichen *Usnea Dasypoga* Rohl

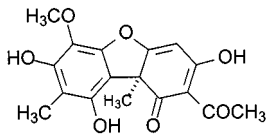
Wahyudi Priyono Suwarso,*[a] Ratna Layla Gani,[a] Karsten Krohn,*[b] and Markus John[b]

Keywords: Natural products / Trilactide / Lichen / Macrocycles / Lactones

A structurally novel 24-membered C_3-symmetrical macrolactone **2a**, composed of three units of 3,7-dihydroxy-2,4-dimethyloctanoic acid, was isolated from the Indonesian lichen *Usnea dasypoga* Rohl.

More than 1.100 medicinal plants are found in Indonesia, but only 750 species show therapeutic effects in clinical trials.[1] In a joint program, we have investigated the chemical constituents of *Usnea dasypoga* Rohl., a species belonging to the fruticose lichen which is used in traditional medicine in Indonesia against tuberculosis, dysentry, and as a diuretic. *U. dasypoga* Rohl. belongs to the division *thallophytae*, the subdivision *lichenes*, the order *ascolichenes*, the family *usneaceae* and the genus *usnea*.[2] Its dark-grey, hairy-like thalli are up to 5 m long and normally attached to the base branches of trees. Its growth is very slow, normally only 1−10 mm per year, and the organism is particularly sensitive to optimum environmental conditions. A moderate and wet climate with much direct sunlight and unpolluted atmosphere − notably free from sulfur dioxide − is required. Thus, this sensitive lichen may be used as an indication for air pollution conditions.

U. dasypoga Rohl. was collected from the tree branches *Celtis phillipinensis Blanco* (family: *ulmaceae*) growing in the Seblat-National Botanical Garden, Mount Kerinci (1.300 m above the sea level), Province of Jambi (center of Sumatra island). The petroleum ether extract of the dried powdered lichen material showed six spots by TLC analysis. Two compounds were isolated in pure form by column chromatography on silica gel. The less polar material (120 mg) was identified as (+)-usnic acid (**1**). This relatively high content of the biologically active usnic acid (**1**)[3] may be correlated with the use of *Usnea dasypoga* Rohl. in traditional medicine.[4]



Scheme 1. Structure of (+)-usnic acid (**1**)

[a] Chemistry Department, Faculty of Mathematics and Science, The University of Indonesia,
Depok 16424, Indonesia
E-mail: wps@makara.cso.ui.ac.id
[b] Fachbereich Chemie und Chemietechnik, der Universität−GH Paderborn,
Warburger Str. 100, D-33098 Paderborn, Germany
E-mail: kk@chemie.uni-paderborn.de

A second new compound was isolated from the polar fraction. This natural product, named dasypogalactone, crystallizes as colorless needles with m.p. 109−110°C. The material is optically active ($[\alpha]_D^{20} = -2.5$, dichloromethane). Two resonances in the IR spectrum at 1728 and 3435 cm^{-1} are characteristic for carbonyl and hydroxy functions, respectively. The molecular formula of $C_{30}H_{54}O_9$, determined by the HR-DCI mass spectrometry, shows a threefold number of carbon and hydrogen atoms detectable in the respective NMR spectra (see Table 1) and the molecule is probably a symmetrical trimer.

Table 1. ^{1}H-NMR and ^{13}C-NMR data ($CDCl_3$) of dasypogalactone (**2a**)

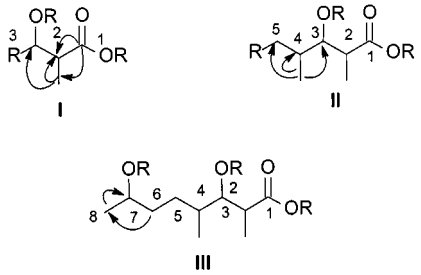
Atom	^{1}H NMR	Multiplicity, no. of atoms (*J*)	^{13}C NMR (multiplicity)
1	−	−	177.05 (s)
2	2.56	m, 1 H	44.10 (d)
2-CH_3	1.09	d, 3 H (7.0 Hz)	14.44 (d)
3	3.61	dd, 1 H (2.4, 8.8 Hz)	77.05 (d)
4	1.54	m, 1 H	34.71 (d)
4-CH_3	0.87	d, 3 H, (6.5 Hz)	12.04 (q)
5	1.35−1.50	m, 2 H	29.12 (t)
6	1.47−1.68	m, 2 H	34.07 (t)
7	5.06	m, 1 H	71.11 (d)
8	1.26	d, 3 H (6.3 Hz)	20.62 (q)
OH	3.23	s, 2 H	−

The signal at δ = 177.05 in the ^{13}C-NMR spectrum confirms the presence of an ester carbonyl group. Analysis of the ^{1}H-NMR spectrum and the DEPT-135 experiment further reveals the presence of three methyl (visible as dublets in the ^{1}H-NMR spectrum), four methine (two of them are monooxygenated as shown by the ^{13}C-NMR chemicals shifts at δ = 71.11 and 77.05), and two methylene groups. In addition, one exchangeable signal is registered in the ^{1}H-NMR spectrum at δ = 3.23.

Three partial fragments **I**−**III** of the monomeric unit can be deduced from the analysis of the HMQC, HMBC, and H,H-COSY spectra. The HMBC spectrum reveals couplings of the signal at δ = 177.05 (ester group, C-1) with the methine group 2-H (δ = 2.56) and 2-CH_3 (δ = 1.09). Starting from the proton resonance of 2-CH_3 a correlation to 2-H is registered in the H,H-COSY spectrum. The signal for 2-H also shows a ^3J connectivity to the proton of the methine group 3-H at δ = 3.61. The chemical shifts of 3-H and

  1434−193X/99/0707−1719 $ 17.50+.50/0

also for C-3 in the ^{1}H-NMR and ^{13}C-NMR spectra are characteristic for a monooxygenation of this methine group. The analysis of further cross signals in the HMBC spectrum between 2-CH_3 and C-2 as well as C-3 allow the construction of partial fragment **I**.

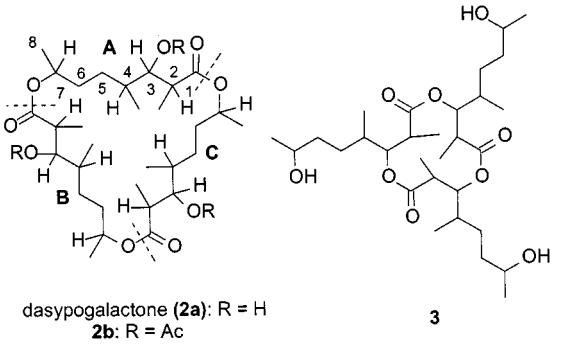


Scheme 2. Partial structures **I**−**III** of the dasypogalactone monomeric unit

Couplings of 2.4 and 8.8 Hz are recorded for the one-proton signal 3-H. The neighborhood to 4-H (δ = 1.54) is shown by a correlation in the H,H-COSY spectrum. This methine group also shows a cross coupling to a three proton doublet at δ = 0.87 (4-CH_3). Further analysis of the ^2J and ^3J couplings in the HMBC spectrum between 4-CH_3 and C-3 and C-4 and the ^{13}C-NMR signal for the methylene group at δ = 29.82 (C-5) leads to partial structure **II**.

Furthermore, the signals for the methylene protons at C-5 show cross signals in the H,H-COSY spectrum to the resonances at δ = 1.47−1.68 for the two protons at C-6 (δ = 34.07). A ^3J connectivity of this carbon resonance to the proton resonances for the methyl group at C-8 (δ = 1.26) can be registered in the HMBC spectrum. In addition, starting from the methyl group at C-8, couplings to the monooxygenated methine group at δ = 5.06 (7-H) are observed in the HMBC and H,H-COSY spectra. Partial structure **III** is finally confirmed by the cross coupling of the proton signal for 7-H to the proton signals at δ = 1.47−1.68 (6-H). Thus, with consideration of one OH signal, a partial fragment of $C_{10}H_{18}O_4RR$ can be deduced. It is reasonable to assume an esterification of the carboxylic group with one of the oxygen atoms in view of the trimeric symmetric nature of the compound. The molecular formula of fragment **III** is thus $C_{10}H_{18}O_3$ and the complete molecule must be the trimer $C_{30}H_{54}O_9$. However, from these deductions it is yet not unequivocally clear which of the two oxygen atoms are involved in the lactone formation resulting in the 24-membered ring **2a** or the 12-membered ring **3**. To decide between these alternatives, 2 mg of the natural product was acetylated to the triacetate **2b**. A significant downfield shift was expected for the ^{1}H-NMR signal attached to the acetoxy group (4-H or 7-H). In fact, the downfield shift of the signal for 4-H from δ = 3.61 to 5.14 confirmed the 24-membered lactone ring **2b**.

Only few naturally occurring C_3-symmetrical macrotriolides are known (e.g. enterobactin[5]) and the 24-membered trilactide dasypogalactone (**2a**) represents a new class of nonclassical macrolides composed of three units of the hitherto unknown 3,7-dihydroxyoctanoic acid. The relative and absolute configurations of **2a** are not yet fully established.



Scheme 3. Structure of dasypogalactone (**2a**) and the 12-membered isomer **3**

This work will be done in conjunction with the total synthesis.

Experimental Section

For general methods and instrumentation see ref.[6] The mass spectra were recorded with a Finigan MAT 8200 (70 eV) instrument. The assignment of the ^{1}H-NMR and ^{13}C-NMR signals were verified by the HMQC spectra.

Isolation: The thalli of *U. dasypoga* Rohl. were first air-dried and then crushed to a fine powder. 308 g of this fine powder was extracted three times with petroleum ether (60 mL). After evaporation of the solvent under reduced pressure, 2.70 g of a crude extract was obtained. TLC analysis of the crude material revealed the existence of six spots. Column chromatography on silica gel (gradients of ethyl acetate/*n*-hexane, 0−16% v/v) yielded from the unpolar fraction 120 mg of a compound identified as (+)-usnic acid (**1**) (yellow needles, m.p. 203°C, ref.[3] m.p. 203°C). The polar fraction was further purified by preparative TLC on silica gel (1 mm, ethyl acetate/5% *n*-hexane) to afford 36 mg of macrolactone **2a** as white crystals, m.p. 109−110°C; R_f = 0.45 (CH_2Cl_2/4% MeOH, detectable by spraying with 8% ethanolic sulfuric acid).

3,7-Dihydroxy-2,4-dimethyloctanoic Acid 7,7′,7′′-Trilactid [Dasypogalactone (2a)]: Colorless needles, m.p. 108−110°C; $[\alpha]_D^{20}$ = −2.5 (c = 0.6; CH_2Cl_2). − IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1}, 1728, 1458, 1375, 1182, 1124. − ^{1}H- and ^{13}C-NMR data see Table 1. − EIMS& SEMI; *m/z* (%): 558 (1.5) $[M]^+$, 540 (2), 530 (2.5), 355 (5), 270 (18), 187 (92), 169 (100), 113 (88), 95 (22). − CIMS [NH_3 (pos.)]; *m/z* (%): 576 (100) $[M + NH_4]^+$, 558 (10) $[M]^+$. − HRDCIMS [NH_3 (neg.)]; $C_{30}H_{53}O_9$: calcd. 557.3767, found 557.3656 $[M - H]^-$ ± 3 ppm.

Dasypogalactone Triacetate (2b): Dasypogalactone (**2a**) (2 mg, 3.6 μmol) was treated with acetic anhydride (0.1 mL), pyridine (0.03 mL), and 4-(dimethylamino)pyridine (DMAP, 1 mg) and the mixture was stirred under argon for 2 h. The solution was then diluted with dichloromethane (2 mL) and extracted three times with 2 N HCl (3 mL). The organic phase was dried ($MgSO_4$) and concentrated at reduced pressure to afford the oily triacetate **2b** (2.2 mg, 90%). − IR (KBr): $\tilde{\nu}$ = 1742 cm^{-1}, 1726, 1273, 1183, 1027 cm^{-1}. − ^{1}H NMR ($CDCl_3$): δ = 0.90 (d, 3 H, J = 6.8 Hz, 4-CH_3), 1.18 (d, 3 H, J = 6.5 Hz, 8-H), 1.20 (d, 3 H, J = 7.1 Hz, 2-CH_3), 1.30−2.00 (m, 5 H, 4-H, 5-H, 6-H), 2.11 (s, 3 H, CH_3CO), 2.83 (m, 1 H, 2-H), 4.90 (m, 1 H, 7-H), 5.15 (dd, 1 H, J = 4.0 Hz, J = 8.2 Hz, 3-H). − HRDCIMS [NH_3 (neg.)]; $C_{36}H_{59}O_{12}$: calcd. 663.3965, found 663.4006 $[M - H]^-$ ± 3 ppm.

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[4] The other component, dasypogalactone (**2a**), did not show antibacterial activity or an inhibition of the endotheline converting enzyme and the protein tyrosine kinase (preliminary tests by Dr. Delzer, BASF, Ludwigshafen).

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Received September 10, 1998
[O98413]