

# Dasypogalactone, a New C<sub>3</sub>-Symmetric Macrolactone from the Indonesian Lichen *Usnea Dasypoga* Rohl

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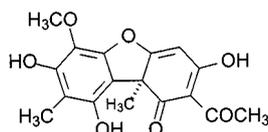
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A structurally novel 24-membered C<sub>3</sub>-symmetrical macrolactone **2a**, composed of three units of 3,7-dihydroxy-2,4-

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.<sup>[1]</sup> 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, dysentery, 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*.<sup>[2]</sup> 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 philippinensis* 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**)<sup>[3]</sup> may be correlated with the use of *Usnea dasypoga* Rohl. in traditional medicine.<sup>[4]</sup>



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

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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  $\text{cm}^{-1}$  are characteristic for carbonyl and hydroxy functions, respectively. The molecular formula of C<sub>30</sub>H<sub>54</sub>O<sub>9</sub>, determined by the HR-DCI mass spectrometry, shows a three-fold number of carbon and hydrogen atoms detectable in the respective NMR spectra (see Table 1) and the molecule is probably a symmetrical trimer.

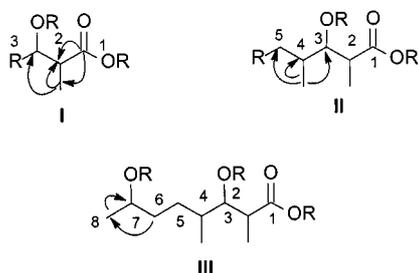
Table 1. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data (CDCl<sub>3</sub>) of dasypogalactone (**2a**)

Atom	<sup>1</sup> H NMR	Multiplicity, no. of atoms ( <i>J</i> )	<sup>13</sup> C NMR (multiplicity)
1	–	–	177.05 (s)
2	2.56	m, 1 H	44.10 (d)
2-CH <sub>3</sub>	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 <sub>3</sub>	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  $\delta = 177.05$  in the <sup>13</sup>C-NMR spectrum confirms the presence of an ester carbonyl group. Analysis of the <sup>1</sup>H-NMR spectrum and the DEPT-135 experiment further reveals the presence of three methyl (visible as doublets in the <sup>1</sup>H-NMR spectrum), four methine (two of them are monooxygenated as shown by the <sup>13</sup>C-NMR chemical shifts at  $\delta = 71.11$  and 77.05), and two methylene groups. In addition, one exchangeable signal is registered in the <sup>1</sup>H-NMR spectrum at  $\delta = 3.23$ .

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  $\delta = 177.05$  (ester group, C-1) with the methine group 2-H ( $\delta = 2.56$ ) and 2-CH<sub>3</sub> ( $\delta = 1.09$ ). Starting from the proton resonance of 2-CH<sub>3</sub> a correlation to 2-H is registered in the H,H-COSY spectrum. The signal for 2-H also shows a <sup>3</sup>*J* connectivity to the proton of the methine group 3-H at  $\delta = 3.61$ . The chemical shifts of 3-H and

also for C-3 in the  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra are characteristic for a monooxygenation of this methine group. The analysis of further cross signals in the HMBC spectrum between 2- $\text{CH}_3$  and C-2 as well as C-3 allow the construction of partial fragment **I**.

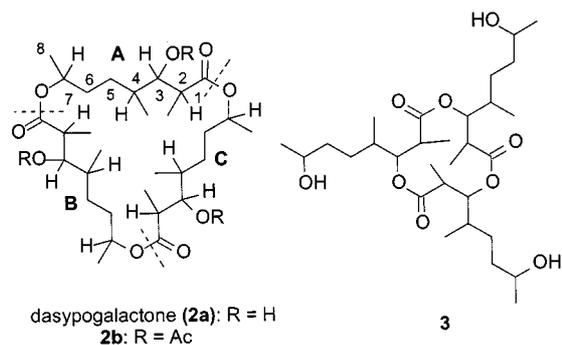


Scheme 2. Partial structures **I–III** of the dasypogalactone monomeric unit

Couplings of 2.4 and 8.8 Hz are recorded for the one-proton signal 3-H. The neighborhood to 4-H ( $\delta = 1.54$ ) is shown by a correlation in the  $\text{H}_2\text{H-COSY}$  spectrum. This methine group also shows a cross coupling to a three-proton doublet at  $\delta = 0.87$  (4- $\text{CH}_3$ ). Further analysis of the  $^2J$  and  $^3J$  couplings in the HMBC spectrum between 4- $\text{CH}_3$  and C-3 and C-4 and the  $^{13}\text{C-NMR}$  signal for the methylene group at  $\delta = 29.82$  (C-5) leads to partial structure **II**.

Furthermore, the signals for the methylene protons at C-5 show cross signals in the  $\text{H}_2\text{H-COSY}$  spectrum to the resonances at  $\delta = 1.47\text{--}1.68$  for the two protons at C-6 ( $\delta = 34.07$ ). A  $^3J$  connectivity of this carbon resonance to the proton resonances for the methyl group at C-8 ( $\delta = 1.26$ ) can be registered in the HMBC spectrum. In addition, starting from the methyl group at C-8, couplings to the monooxygenated methine group at  $\delta = 5.06$  (7-H) are observed in the HMBC and  $\text{H}_2\text{H-COSY}$  spectra. Partial structure **III** is finally confirmed by the cross coupling of the proton signal for 7-H to the proton signals at  $\delta = 1.47\text{--}1.68$  (6-H). Thus, with consideration of one OH signal, a partial fragment of  $\text{C}_{10}\text{H}_{18}\text{O}_4\text{RR}$  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  $\text{C}_{10}\text{H}_{18}\text{O}_3$  and the complete molecule must be the trimer  $\text{C}_{30}\text{H}_{54}\text{O}_9$ . 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  $^1\text{H-NMR}$  signal attached to the acetoxy group (4-H or 7-H). In fact, the downfield shift of the signal for 4-H from  $\delta = 3.61$  to 5.14 confirmed the 24-membered lactone ring **2b**.

Only few naturally occurring  $\text{C}_3$ -symmetrical macrotrioles are known (e.g. enterobactin<sup>[5]</sup>) 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.<sup>[6]</sup> The mass spectra were recorded with a Finigan MAT 8200 (70 eV) instrument. The assignment of the  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  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.<sup>[3]</sup> 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;  $R_f = 0.45$  ( $\text{CH}_2\text{Cl}_2/4\%$  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;  $[\alpha]_{\text{D}}^{20} = -2.5$  ( $c = 0.6$ ;  $\text{CH}_2\text{Cl}_2$ ). – IR (KBr):  $\tilde{\nu} = 3435\text{ cm}^{-1}$ , 1728, 1458, 1375, 1182, 1124. –  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data see Table 1. – EIMS& SEMI;  $m/z$  (%): 558 (1.5)  $[\text{M}]^+$ , 540 (2), 530 (2.5), 355 (5), 270 (18), 187 (92), 169 (100), 113 (88), 95 (22). – CIMS  $[\text{NH}_3$  (pos.);  $m/z$  (%): 576 (100)  $[\text{M} + \text{NH}_4]^+$ , 558 (10)  $[\text{M}]^+$ . – HRDCIMS  $[\text{NH}_3$  (neg.);  $\text{C}_{30}\text{H}_{53}\text{O}_9$ : calcd. 557.3767, found 557.3656  $[\text{M} - \text{H}]^- \pm 3$  ppm.

**Dasypogalactone Triacetate (**2b**):** Dasypogalactone (**2a**) (2 mg, 3.6  $\mu\text{mol}$ ) 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 ( $\text{MgSO}_4$ ) and concentrated at reduced pressure to afford the oily triacetate **2b** (2.2 mg, 90%). – IR (KBr):  $\tilde{\nu} = 1742\text{ cm}^{-1}$ , 1726, 1273, 1183, 1027  $\text{cm}^{-1}$ . –  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta = 0.90$  (d, 3 H,  $J = 6.8$  Hz, 4- $\text{CH}_3$ ), 1.18 (d, 3 H,  $J = 6.5$  Hz, 8-H), 1.20 (d, 3 H,  $J = 7.1$  Hz, 2- $\text{CH}_3$ ), 1.30–2.00 (m, 5 H, 4-H, 5-H, 6-H), 2.11 (s, 3 H,  $\text{CH}_3\text{CO}$ ), 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  $[\text{NH}_3$  (neg.);  $\text{C}_{36}\text{H}_{59}\text{O}_{12}$ : calcd. 663.3965, found 663.4006  $[\text{M} - \text{H}]^- \pm 3$  ppm.

- [1] N. R. Farnsworth, *The Napralet Data Base as Information Sources for Application to the Traditional Medicine*, in: *Traditional Medicine and Health Care Coverage*, WHO, Geneva, **1983**.
- [2] [2a] G. Tjitrosupono, *The Botanical Taxonomy of Thallophyte, Bryophyte and Pteridophyte*, Gadjah Mada University Press, Yogyakarta, Indonesia, **1994**. – [2b] C. F. Culberson, *Chemical and Botanical Guide to Lichen Products*, Otto Koeltz Science Publisher, Koenigsstein, **1979**, p. 538–539. – [2c] C. F. Culberson, W. L. Culberson, A. Johnson, *Second Supplement to Bryological and Lichenological Society*, American Bryological and Lichenological Society, Ann Arbor, Michigan, USA, **1997**, p. 345.
- [3] *Römpp Lexikon Naturstoffe* (Ed.: W. Steglich, B. Fugmann, S. Lang-Fugmann), Georg Thieme, Stuttgart, New York, **1997**, p. 675.
- [4] The other component, dasypogalactone (**2a**), did not show antibacterial activity or an inhibition of the endothelium converting enzyme and the protein tyrosine kinase (preliminary tests by Dr. Delzer, BASF, Ludwigshafen).
- [5] J. B. Neilands, *Ann. Rev. Microbiol.* **1982**, *36*, 285–309.
- [6] K. Krohn, A. Michel, U. Flörke, H.-J. Aust, S. Draeger, B. Schulz, *Liebigs Ann. Chem.* **1994**, 1093–1097.

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