# DASYPOGALACTONE, A NEW CHIRALIC C<sub>3</sub>-SYMMETRIC MACROLACTONE FROM LICHEN USNEA DASYPOGA ROHL.

Ratna Layla Gani\*, Wahyudi Priyono Suwarso\*\*, Karsten Krohn\*\*\*and Markus John

\*Faculty of Pharmacy, the Pancasila University, Jagakarsa, Jakarta \*\*Departement of Chemistry, Faculty of Mathematics and Sciences, the University of Indonesia, Jakarta. \*\*\*Fachbereich Chemie und Chemietechnik, der Universitat-GH Paderborn, Warburger Str. 100, D-33098 Padaborn, Germany.

## ABSTRACT

Medicinal plants spread out in Indonesia, however only a part of them have been clinically investigated that exhibited therapeutic effecs. This research was to isolate a new compounds in *Usnea dasypoga*.

A new twentyfour-membered macrolactone composed of three units of unknown fatty acid  $(2R^*, 3R^*, 4S^*, 7S^* \text{ or } 2S^* 3S^* 4R^*, 7R^*)$ -3,7-dihydroxy-2,4-dimethyloctanoic acid forming a C<sub>3</sub>-symmetrical lactide was isolated from Indonesian *lichen Usnea dasypoga* Rohl.

The result shown that those compound had been confirmed with the spectroscopic data.

Keywords: Natural products isolation, three lactide, lichen

## ABSTRAK

Tanaman *Usnea dasypoga* tersebar luas di Indonesia tetapi hanya sebagian dari mereka yang telah diteliti secara klinik mempunyai efek terapi. Penelitian ini untuk menemukan senyawa baru dari *Usnea dasypoga*.

Dalam penelitian ini telah ditemukan senyawa baru dari anggota makrolakton yang terdiri dari 24 unit lakton dan 3 unit asam lemak yang tidak diketahui (2R\*, 3R\*, 4S\*, 7S\* atau 2S\*, 3S\*, 4R\*, 7R\*) – 3,7- dihidroksi-2,4-dimetil-asam oktanoat yang membentuk laktida yang simetris dari  $C_3$ .

Hasil menunjukkan bahwa senyawa tersebut telah sesuai dengan data hasil analisis spektroskopis. **Kata Kunci** : Isolasi produk alami, trilaktida, lumut (Usnea)

## **INTRODUCTION**

More than 1.100 medicinal plants can be found in Indonesia, but only 750 species are known to have therapeutic effects by clinical trial (Farnsword, 1983). In this work, we have investigated the chemical constituents of *Usnea dasypoga* Rohl., a species belonging to frutocose lichen which is used in traditional medicine in Indonesia. *Usnea. dasypoga* Rohl. belongs to the division *thallophyte*, the subdivision *lichenes*, the order *ascolichenes*, the family *usneaceae* and the genus *usnea* (Tjitrosupono, 1994; Culberson, 1979; Culberson *et al.*, 1977). Its dark-grey thalli hairy-like are up to 5.0 m long and are normally attached at the base branches of trees. Its growth is very slow, normally only 1-10 mm per year, and the organism is particularly sensitive to optimal environmental conditions. A moderate and wet climate with much direct sun irridiation and unpolluted air, notably free of sulfur dioxide, is required. Therefore, this sensitive lichen might used for air pollution control.

## **METHODS**

## General

For general methods and instrumentation see Krohn, Michel, Florke, Aust, Draeger, Schulz (1994) The mass spectra were recorded with a Finigan MAT 8200 (70 eV) instrument. The assignment of the 'H- and <sup>13</sup>C- NMR signals were verified by the HMQC spectra.

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#### **Plant Material**

Thallus of *Usnea dasypoga* Rohl was collected from the tree branches of *Celtis phillipinensis* Blanco (family : *ulmaceae*) growing in the Seblat-National Botanical Garden, Mount Kerinci (1.300 m above the sea level), Province of Jambi (central of Sumatra island). The dried thallus of the *Usnea dasypoga* Rohl. was kept at the Department of Chemistry, Faculty of Matematics and Sciences, the University of Indonesia Jakarta. It was identified by LIPI-Botanical Laboratory, Bogor.

#### **Extraction and Isolation**

The thalli of *U. dasypoga* Rohl. were firstly air dried and then crushed to fine powder, 308 g of this fine powder was extracted three times with petroleum ether using Soxhlet apparatus (60 mL). Solvent was evaporated under reduced pressure, 2.70 g of raw extract was obtained. The content of substances in the crude material were analyzed by TLC using stationary phase silica gel G and eluent  $CH_2C1_2 - MeOH$ , (4:96) and appeared spots were detected under UV-lamp, and then was sprayed with 8% ethanolic sulfuric acid. Isolation of pure substances were done by column chromatography on silica gel (*n*-hexane-ethylacetate).

#### **RESULTS AND DISCUSSION**

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 (**la**). This compound is yellow crystal with melting point = 204-205 °C (Steglich , *et al.*, 1997,: m.p. 203 °C) and has specific optical rotation,  $[\alpha]_D^{20} = + 493^\circ$  (c = 0.2, CHCI<sub>3</sub>) (Layla-Gani, 1998) This relatively high content of the biologically active (+)-usnic acid (**la**) might be correlated to the use of *Usnea dasypoga* Rohl. in traditional medicine (Steglich, *et al.*, 1997).

The recently result from the X-ray diffraction of (+)-usnic acid crystal (la), Krohn, *et al.*, (2000) showed, that instead on up now known only two in cyclohexane-ringfom carbonyl-hydroxyl hydrogen bonds, actually (+)usnic acid (la). The isolated compound has one more but in cycloheptane-ringform carbonyl-hydroxyl hydrogen bond (lc) (Figure 2).

This additionally hydrogen bond is proposed weaker than other hydrogen bond, because it is formed from the seven membered ring. The ring A and ring C of (+)-usnic acid (la) is not coplanar with ring B, but there are bending in same direction and opposite with the position of methyl group attached at the asymmetric carbon atom. Consequently, the distance between ring A and ring C of (+)-usnic acid (la) became shorter. As the result, the hydrogen atom of hydroxyl group at the ring A can form the hydrogen bond with keto group at ring C although in form of seven ring (fig. lb.). However, it is reasonable to assume, that the (+)-usnic acid (la) as well as (-)usnic acid (lb) has a great specific optical rotation  $[\alpha]_D^{20} = +500^\circ$  or  $-500^\circ$ .

Figure. 1. Molecular structure of (+)-usnic acid la and (-)-usnic acid lb

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Figure 2. Molecular stereochemical structure of (+)-usnic acid (lc) and its X-ray structure

A second new compound was isolated from the polar fraction This natural product is, named dasypogalactone, crystallized as colorless needles with m.p. 109-110 °C and this compound is optically active  $[\alpha]_D{}^{20}$  = -2.5°, c = 0.6, CH<sub>2</sub>Cl<sub>2</sub>. Two resonances in the IR spectrum at v = 1728 and 3435 cm<sup>-1</sup> are charasteristic for carbonyl and hydroxy function, respectively. The molecular formula of C<sub>30</sub>H<sub>54</sub>O<sub>9</sub>, determined by the HR-DCI mass spectrum, shows a trifold number of carbon and hydrogen atoms detectable in the respective NMR spectra (Tabel I).

The signal at  $\delta = 177.05$  ppm in the <sup>13</sup>C-NMR spectrum confirms the presence of an ester carbonyl group. Analysis of the 'H-NMR spectrum and the DEPT-135 experiment further reveals the presence of three methyl (visible as doubletts in the 'H-NMR spectrum), four methine (two of which are monooxygenated as shown by the <sup>13</sup>C-NMR chemicals shifts at  $\delta = 71.11$  and 77.05 ppm), and two methylene groups. In addition two exchangeable signals are registered in the 'H-NMR spectrum at  $\delta = 3.23$  and 3.97 ppm.

Atom	<sup>1</sup> H-NMR,	m*, No. of atoms,	$^{13}$ C-NMR(m*)
No.	ppm	coupling constant $(J)$	ppm
1	-	-	177.05 (s)
2	2.56	m, 1 H	44.10 (d)
2-CH <sub>3</sub>	1.09	d,3H (7.0 Hz)	14.44 (d)
3	3.61	dd, 1 H (2.4 and 8.8	77.05 (d)
4	1.54	Hz)	34,71 (d)
$4-CH_3$	0.87	m, 1 H	12.04 (q)
5	1.35-1.50	d,3H, (6.5 Hz)	29,12 (t)
6	1.47-1.68	m, 2 H	34.07 (t)
7	5.06	m, 2 H	71.11 (d)
8	1.26	m, 1 H	20.62 (q)
OH	3.23	d, 3H (6.5 Hz)	-
OH	3.97	s, 2 H s, 1 H	-

Tabel I. 'H-NMR and <sup>13</sup>C-NMR data of dasypogalactone 2a (measured in CDC1<sub>3</sub>)

\* = multiplicity

Three partial fragments I-III can be deduced from the analysis of the HMQC, HMBC and H,H-COSY spectra. The HMBC spectrum reveals coupling of the signal at  $\delta = 177.05$  pprn (ester group, C-1) with a methine group 2-H ( $\delta = 2.56$  ppm) and 2CH<sub>3</sub>( $\delta = 1.09$  pprn). 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 medthine group 3-H at  $\delta = 3.61$  ppm. The chemical shifts of 3-H and also for C-3 in the 'H - and <sup>13</sup>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<sub>3</sub> and C-2 as well as C-3 allow the construction of partial fragment I (Figure 3).

Figure. 3.. Partial structures I - III of the dasypogalacton monomeric unit

Couplings of  ${}^{3}J$  =2.4 and 8.8 Hz are recorded for the one proton signal for 3-H. The neighbourhood to 4-H ( $\delta$  = 1.54 ppm) is shown by a correlation in the H,H-COSY spectrum.

This methine group also shows a cross coupling to a three proton doublett at  $\delta$ =0.87 ppm (4-CH<sub>3</sub>). Further analysis of the <sup>2</sup>J and <sup>3</sup>J coupling in the HMBC spectrum between 4-CH<sub>3</sub> and C-3 and C-4 and the, <sup>13</sup> C-NMR signal for the methylene group at  $\delta$  = 29.82 ppm (C-5) leads to partial structure II (Figure 3).

Furthermore, the signal for the methylene protons at C-5 show cross signals in the H,H-COSY spectrum to the resonances at  $\delta = 1.47$ -1.68 ppm for the two protons at C-6 ( $\delta = 34.07$  ppm). A <sup>3</sup>J 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, coupling to the monooxygenated methine group at  $\delta = 5.06$  ppm (7-H) are observed in the HMBC and H,H-COSY spectra.

Partial structure III (Figure 3) is finally confirmed by the cross coupling of the proton signal for 7-H to the proton signals at  $\delta = 1.47$ -1.68 ppm (6-H). Thus, with consideration of one OH signal, a partial fragment of  $C_{10}H_{18}0_4R_1R_2$  can be deduced. It is reasonable to assume an esterification of the carboxylic group with one of the oxygens in view of the trimeric symmetric nature of the compound. The molecular formula for fragment III (Figure 3) is  $C_{10}H_{54}0_3$  and the entire rnolecule must be the trimer  $C_{30}H_{54}0_9$ .

However, from these deductions it is yet clear which of the two oxygens are involved in the lactone formation resulting in a 24-membered ring 2a or the 12-membered ring 3 (Figure 4). To decide between these alternatives, 2. 0 mg of the natural product were acetylated to the be triacetate 2b. A significant downfield shift was expected for the proton NMR signal attached to the acetoxy group (4-H or 9-H). In fact, the downfield shift of the signal for 4-H from 3.61 to 5.14 ppm unequivocally confirmed the 24-membered lactone ring 2b. Also the 24-membered trilactide dasypogalactone 2a represents a new class of non-classical macrolides composed of three units of the hitherto unknown 3,7-dihydroxyoctanoic acid.

The relative configuration of the four chiral centers in the identical three fragments in 2a was achieved by the analysis of the coupling constants and NOE difference spectra of the conformationally rigid trilactone. To facilitate the NOE discussion the three hydroxy octanoic acids as fragments A, B, and C are assigned in formula 2a. A key information was obtained from the coupling constants of  $J_{2,3} = 8,8$  Hz and  $J_{3,4} = 2.4$  Hz establishing the 2,3 *–anti* and the *3,4-syn* configuration of the respective protons as shown in the stereoformula 2a. This assignment was confirmed by NOE difference spectra showing interactions of 4-CH<sub>3</sub> with 2-H (8.6 %) and of 2-CH<sub>3</sub> with 4-H (4 %) establishinh the *syn* relation of thew groups. Finally, the configuration of the distant stereogenic of the center at C-7 was elucidated by the NOE interaction 4-CH<sub>3</sub> with 2-H.

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Figure 4. Stereochemical structure of dasypogalactone2a ; R=H, dasypogalactone with 2S\*,3S\*,4R\*,7R\*2a : R=H, dasypogalactone with 2S\*,3S\*,4R\*,7R\*2b : R=acetyl group2b; R=acetyl group

Figure 5. Two possibilities of stereochemical structure of dasypogalactone 2a and its the 12-membered proposed isomer

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IV: 2S\*,3S\*,4R\*,7R\* V:2R\*,3R\*,4S\*,7S\* Figure 6. Two possibilities of relative configuration of dasypogalactone monomeric unit of 2a (Figure 4)

The C-8 methyl group with 2-H (1.9 %) and 3-H (1.7 %) of the neighbouring carboxylic acid and the 3,4-syn configuration of the respective protons as shown in t.he stereoformula 2a (Figure 4). This assignment was confirmed by NOE difference spectra showing interactions of 4-CH<sub>3</sub> with 2-H (8.6 %) and of 2-CH<sub>3</sub> with 4-H (4 %) establishing the *syn* relation of these groups. Finally, the configuration of the distant stereogenic center at C-7 was elucidated by the NOE interaction of the C-8 methyl group with 2-H (1.9 %) and 3-H (1.7 %) of the neighbouring carboxylic acid fragment B. According to model considerations these interactions are only possible with 2,8-syn and 3,8-anti configuration of the appropriate protons as shown in the stereoformula 2a. Also from these above conformational analyses can be proposed two possibilities of relative configuration of dasypogalactone :  $2S^*$ ,  $3S^*$ ,  $4R^*$ ,  $7R^*$  or  $2R^*$ ,  $3R^*$ ,  $4S^*$ ,  $7R^*$  (2a).

#### CONCLUSION

A new compound that has twenty four units lactone as macrolactone (dasypogalactone) had been isolated, and three units of lactone might have two stereochemical structure configurations;  $2S^*, 3S^*, 4S^*$ ,  $7R^*$  or  $2R^*, 3R^*, 4S^*, 7R^*$ .

### REFERENCES

- Culberson, C.F., Culberson W.L., Johnson, A. (1977), Second Supplement to Bryological and Lichenologica Society, American Bryological Society and Lichenological Society, Ann Arbor, Michigan. USA, p. 345
- Culberson, C.F. (1979), *Chemical and Botanical Guide to Lichen Products*, Otto Koelten Science Publisher, Koenigstein, Germany, p. 538 - 539
- Farnsworth N.R. (1983) The Napralet Data Base as Information Sources for Application to the Traditional Medicine: in Traditional Medicine and Health Care Coverage, WHO, Geneve.
- Krohn, K., Michel A., Florke, U., Aust, H.J., Draeger, S., B. Schulz, B.(1994)Liebigs Ann. Chem., 1093-1097.
- Krohr, K, Steingroever K, Florke, U., (2000), personal communication
- Layla-Gani, R. (1998) Master Thesis, Departmen of Chemistry., Fac. of Mathematics and Science, the University of Indonesia, Jakarta, Indonesia.
- Steglich, W., Fugmann, B., Lang-Fugmann, S., Eds. (1997), *Rompp Lexikonu.Naturstoffe* Georg Thieme, Stuttgart, New York, p. 675
- Tjitrosupono, G. (1994) *The Botanical Taxonomy of Thallophyte, Bryophyte and Pteridophyte,* Gadjah Mada University Press, Yogyakarta Indonesia,