Phytochemistry, 1979, Vol. 18, pp. 325–326. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0325 $02.00/0

SYNTHESIS OF NARTHOGENIN, THE AGLYCONE OF NARTHECIDE

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(*Received* 2 *August* 1978)

Key Word Index—*Narthecium ossifragum*; Liliaceae; narthogenin; antibiotic α-lactone; tetronic acid derivatives; synthesis.

Abstract—A synthetic route to obtain 5-hydroxy-4-methoxy-2,5-dihydrofuran-2-one, is described and spectral data obtained for this compound are compared with those of narthogenin, the racemic aglycone of narthecide A and B. The compounds were proved to be identical.

Narthecium ossifragum has previously been investigated [1, 2] and among other compounds a new antibiotic glycoside, named narthecide was isolated. The aglycone of narthecide was shown to possess either structure **1** or **2**. No definite destinction between the two structures was possible at that time. Later, a reinvestigation [3] of *N. ossifragum* showed that narthecide consisted of two diastereomers, narthecide A and B. On enzymatic hydrolysis of narthecide A and B, as well as by direct extraction of the plant, a racemic, antibiotic γ-lactone was isolated and structure **2** was assigned to the compound on the basis of its spectral data.

The published ^{1}H NMR chemical shift value of the methoxy group in authentic narthogenin seemed somewhat anomalous when compared to the corresponding value found for 4-*O*-methyltetronic acid (**3**) (Table 1). This apparent anomaly, together with the fact that compound **2** had not so far been synthesized, made it of interest for the unambiguous structure assignment of narthogenin to synthesize **2** and compare the NMR data with those obtained for narthogenin. Further, a synthetic route to **2** should make it possible to perform a more extensive biological and medical testing of the compound.

As a starting point for the synthesis, compound **3** [4, 5] was chosen. Treatment of **3** with one equivalent of *N*-bromosuccinimide gave a mixture of three brominated compounds which could easily be separated and identified as the compounds **4**, **5** and **6**, compound **4** being the major product. On reaction of **5** with silver oxide in water, compound **2** was formed. The IR spectra of **2** and authentic narthogenin were identical.

For comparison, an authentic sample of narthecide was enzymatically hydrolysed to narthogenin and the NMR data for the latter compound showed a close correspondence to those found for **2** (Table 1). Thus it can be concluded that narthogenin actually has structure **2** and that the NMR data previously reported [3], apparently caused by some systematical error, are no longer tenable. It also seems reasonable to conclude that narthogenin (**2**) is present as the hemiacetal (lactol) in aqueous solution (*cf.* the NMR data for **2** in $CDCl_3$ and in D_2O).

Table 1. NMR spectra of some derivatives of 4-*O*-methyltetronic acid

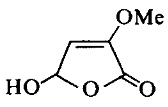
Compound	H-3	OCH_3	H-5
3*	5.10	3.94	4.64
4*	—	4.20	4.68
5*	5.20	4.00	6.60
6*	—	4.40	6.67
2†	5.32	3.93	6.01
2*	5.09	3.93	5.87
Authentic narthogenin*	5.12	3.92	5.91
Authentic narthogenin[3]	5.84	4.45	6.53

Chemical shifts (δ) in ppm; all signals are singlets.
* In $CDCl_3$ with TMS as internal standard. † In D_2O with dioxan as internal standard.

EXPERIMENTAL

MS were recorded using the direct sample insertion system with the lowest feasible sample temp. and ionisation by electron impact (70 eV). Mps are uncorr.

Bromination of 4-methoxy-2,5-dihydrofuran-2-one (*4-O-methyltetronic acid*) (**3**). Compound **3** [4, 5] (0.25 g, 0.0022 mol) was treated for 1 hr in refluxing tetrachloromethane (20 ml) with *N*-bromosuccinimide (0.39 g, 0.0022 mol) and dibenzoyl peroxide as initiator. The solid obtained upon filtration of the cooled soln was washed with 2 N NaOH to remove the succinimide formed. The remaining solid was washed with H_2O and dried to give 0.19 g of pure *3-bromo-4-methoxy-2,5-dihydrofuran-2-one* (*3-bromo-4-O-methyltetronic acid*) (**4**) as colourless crystals, mp 111–114°. NMR: Table 1. MS *m/e* (rel. int.): 194 (45), 192 (50), 165 (100), 163 (90), 149 (12), 147 (12), 83 (25). High resolution MS measurement: 191.94552 (calcd for $C_5H_5O_3$ ^{79}Br: 191.94219).



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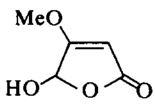

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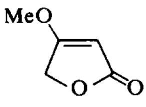

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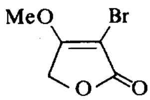

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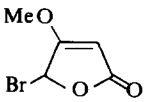

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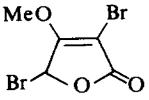

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The tetrachloromethane soln was concd *in vacuo* to give a brown oil which was separated by PLC (Si gel, $CHCl_3$ as eluent). Three fractions contained brominated compounds and they were characterized as follows: $R_f = 0.12$: 0.04 g of colourless crystals which showed MS and NMR identical to the values found for **4**. The total yield of **4** was 54%.

$R_f = 0.26$: 0.04 g (9%) of *5-bromo-4-methoxy-2,5-dihydrofuran-2-one* (*5-bromo-4-O-methyltetronic acid*) (**5**) as a brown oil. NMR: Table 1. MS *m/e* (rel. int.): 194 (3), 192 (3), 113 (100); 85 (21), 69 (25). High resolution MS measurement: 191.94407 (calcd for $C_5H_5O_3$ ^{79}Br: 191.94219).

$R_f = 0.43$: 0.03 g (5%) of *3,5-dibromo-4-methoxy-2,5-dihydrofuran-2-one* (*3,5-dibromo-4-O-methyltetronic acid*) (**6**) as a brown oil. NMR: Table 1. MS *m/e* (rel. int.): 274 (10), 272 (23), 270 (12), 193 (100), 191 (100), 155 (12), 153 (12), 122 (13), 120 (13), 83 (15), 59 (35). High resolution MS measurement: 269.85658 (calcd for $C_5H_4O_3^{79}Br_2$: 269.85270).

5-Hydroxy-4-methoxy-2,5-dihydrofuran-2-one(5-hydroxy-4-O-methyltetronic acid) (**2**). The crude compound (**5**) (0.04 g, 0.002 mol) was treated in 2 ml H_2O with Ag_2O (0.025 g, 0.001 mol) for 20 min. The formed AgBr was allowed to deposit, the aq. phase was isolated, and the AgBr was thoroughly washed with H_2O. The combined aq. phases were evapd to dryness to give 0.02 g colourless crystals. Recrystallization from Et_2O afforded crystals 0.01 g (38%) of mp 137–138.5°. NMR: Table 1. MS *m/e* (rel. int.): 130 (31), 85 (18), 84 (40), 69 (100). IR identical to that given for authentic narthogenin.

Acknowledgements—The authors are grateful to Dr. Arnulv Stabursvik, Department of Chemistry, Agricultural College of Norway, who kindly supplied us with an IR spectrum of authentic narthogenin as well as with an authentic sample of narthecide.

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Phytochemistry, 1979, Vol. 18, pp. 326–327. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0326 $02.00/0

STRUCTURE OF LEPIOCHLORIN, AN ANTIBIOTIC METABOLITE OF A FUNGUS CULTIVATED BY ANTS

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(*Revised received* 6 *September* 1978)

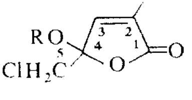
Key Word Index—*Lepiota* species; Basidiomycetes; *Cyphomyrmex costatus*; leaf-cutting ants; Attines; gardening ants; fungal metabolites; lepiochlorin.

A new antibacterial lactol, lepiochlorin, was isolated from liquid cultures of a *Lepiota* species [1], a fungus cultivated by the gardening ants. On the basis of the data presented here, we assign structure **1** for lepiochlorin.

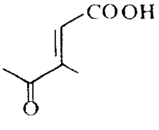
Lepiochlorin, mp 68–70°, did not show a molecular ion in the high resolution EI-MS. It showed intense peaks at *m/e* 113.0228 (M-CH_2Cl; calc. for $C_5H_5O_3$, 113.0238), 85.0303 (113 − CO; calc. for $C_4H_5O_2$, 85.0292). It also showed a weak peak at *m/e* 145.0063 (M − OH; calc. for $C_6H_6ClO_2$, 145.0056). To obtain the correct molecular weight, the chemical ionization spectrum was taken, which showed the highest mass peak at *m/e* 163 (M + H) and the corresponding Cl − 37 isotope peak at *m/e* 165. The high resolution data combined with the chemical ionization results established the molecular formula of lepiochlorin as $C_6H_7ClO_3$. It showed strong end absorption, but no maximum, in the UV spectrum and had ν_{max} at 3250, 1754 and 1670 cm^{-1}. The ^1H NMR spectrum showed a doublet at δ 1.95 (3H, $J = 1.5$ Hz) for the methyl, a singlet at 3.75 (2H) for the methylene, a broad band at 5.1 (1H) for the hydroxyl, and a quartet at 6.95 (1H, $J = 1.5$ Hz) for the olefinic proton. The ^{13}C NMR spectrum showed signals at δ 10.13 (CH_3), 46.87 (C-5), 103.69 (C-4), 134.88 (C-2), 145.26 (C-3) and 172.24 (C-1). Thus, the spectral data of lepiochlorin are consistent only with the δ lactol structure **1**.

Lepiochlorin formed a monoacetate (**2**) which showed ^1H NMR signals at δ 1.98 (3H, *d*, $J = 1.5$ Hz), 2.1 (3H, *s*), 3.8 and 4.11 (2H, AB *q*, $J = 11$ Hz) for the two methylene protons, and 7.16 (1H, *q*, $J = 1.5$ Hz) for the olefinic proton. The CI-MS gave twin peaks at *m/e* 205 and 207.

The alternate structure with methyl on C-3 was eliminated by comparison of the ^1H NMR data of **3** and **1** with those of compounds **4** and **5**, respectively. Compound **4** was prepared from *trans*-3-methyl-4-oxo-2-pentenoic acid. In compound **4** the olefinic proton appeared at δ 5.9 and in compound **5** at 7.19 [2]. The chemical



1 R = H
2 R = Ac



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