Citropremide and Citropridone: A New Ceramide and a New Acridone Alkaloid from the Stem Bark of *Citropsis gabunensis*

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The structure elucidation and complete ^{1}H- and ^{13}C-NMR assignments are reported for two new compounds: the ceramide citropremide (**1**), and the acridone alkaloid citropridone (**2**). Both of these secondary metabolites were isolated from the stem bark of *Citropsis gabunensis*. High-resolution mass, IR and UV spectrometry, and NMR experiments including COSY, HMQC, and HMBC were used for the determination of the structures and NMR spectral assignments.

Introduction. – Acridones (=acridin-9(10*H*)-ones) are ketones of tricyclic molecular-skeleton parents having an N-atom at the 10-position and the keto group at the 9-position. Acridones are known to be capable of having a variety of biological activities, *e.g.*, antiviral, antitumor, antimalarial, and antibiotic properties [1][2]. Acridone derivatives are widespread in nature, commonly occurring in a number of genera of the family Rutaceae [3]. The planar structure of these acridones facilitates their action on nucleotides by intercalating DNA and RNA strands, thereby emerging as potent anticancer agents. Ceramides play an important role in many fields of biochemistry. While their function as structural lipids in membranes, in the epidermis, hair, and nails of humans and animals has been known for a long time, recently the interest has focused on their function as signal transducers [4]. In the course of phytochemical studies of medicinal plants from Africa [5–10], we now investigate *Citropsis gabunensis* (ENGL.) SWINGLE & M. KELLERM. (Rutaceae) and report on the structure elucidation and NMR assignments of two new compounds, namely, citropremide[1]) (**1**), a ceramide, and citropridone (**2**), an acridone alkaloid (*Fig. 1*). High-resolution electron-impact mass spectrometry (HR-EI-MS), IR and UV spectrometry, and one- and two-dimensional NMR experiments were used to determine their structures and NMR spectral assignments.

Results and Discussion. – Citropremide (**1**) was obtained as a colorless powder and was assigned the molecular formula $C_{42}H_{85}NO_5$ on the basis of HR-EI-MS. A ceramide skeleton was indicated by the presence of the following signals in the ^{1}H-NMR

[1]) Arbitrary atom numbering; for the systematic name, see *Exper. Part.*

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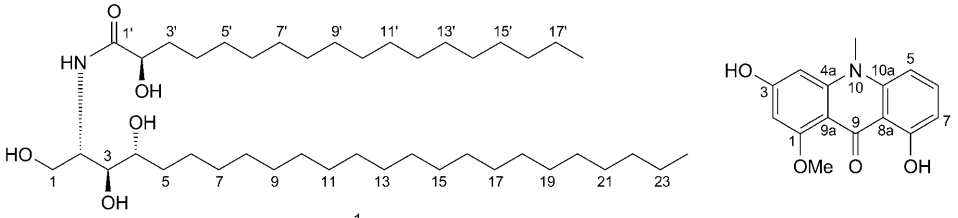

1[1]) **2**

Fig. 1. *Citropremide* (**1**) *and citropridone* (**2**), *isolated from* Citropsis gabunensis

spectrum[1]): δ(H) 0.85 (*t*, $J = 6.5$ Hz, Me(24), Me(18′)) for two terminal Me groups, δ(H) 1.25–1.90 for H-atoms of long CH_2 chains, and δ(H) 8.55 (*d*, $J = 8.8$ Hz) for an amide NH group. In agreement with this were a signal of an NH-attached CH group at δ(C) 53.5 (C(2)) in the ^{13}C-NMR spectrum and a signal of a C=O group at δ(C) 175.7, as well as a strong absorption band at 1640 cm^{-1} for a secondary-amide group in the IR spectrum [5–15]. The ^{1}H-NMR spectrum ((D$_5$)pyridine; *Table*) exhibited five downfield signals assignable to H-atoms geminal to O-bearing groups. Three resulted from CH–O groups (δ(H) 4.62 (*dd*, $J = 4.0$, 8.0 Hz, H–C(2′)), 4.34 (*dd*, $J = 4.5$, 6.5 Hz, H–C(3)), and 4.26–4.32 (*m*, H–C(4))), and two were due to a CH_2O group (δ(H) 4.51 (*dd*, $J = 4.5$, 10.5 Hz, H_a–C(1)) and 4.41 (*dd*, $J = 4.5$, 10.5 Hz, H_b–C(1))). The presence of O-bearing C-atoms at δ(C) 77.4 (*d*), 73.5 (*d*), 73.0 (*d*), and 62.5 (*t*) in the ^{13}C-NMR spectrum established the presence of four OH groups in compound **1**, which were further supported by the absorption band at 3610 cm^{-1} for OH group(s) in the IR spectrum. The absence of a H-atom *t* at *ca.* δ(H) 2.2–2.5 and the appearance of a 1 H *m* at δ(H) 5.09–5.13 in the ^{1}H-NMR spectrum suggested the presence of a C(=O)CH(OH)CH_2 moiety in **1** [15]. The remaining CH_2 H-atoms were overlapped in the region δ(H) 1.25–1.90. In addition to C-atom signals for several CH_2 groups at δ(C) 26.3–34.7, the terminal CH_2 C-atom at δ(C) 23.4 and the terminal Me groups of aliphatic chains at δ(C) 14.7 indicated the absence of any branching [15]. The position of the OH groups were ascertained by the mass fragmentation pattern (*Fig. 2*) and the ^{1}H,^{1}H-COSY and the HMBC data (*Fig. 3*). Cross-peaks in the ^{1}H,^{1}H-COSY plot were observed between the amide H-atom (δ(H) 8.55) and H–C(2) (δ(H) 5.09–5.13), which, in turn, was coupled to three H-atoms at δ(H) 4.51 (H_a–C(1)), 4.41 (H_b–C(1)),

Table. *^{1}H-NMR Data* (C$_5$D$_5$N) *of Citropremide* (**1**) *and of a Synthetic Ceramide.* δ in ppm, *J* in Hz.

	Citropremide (**1**)	Synthetic ceramide[a])
H_a–C(1)	4.51 (*dd*, $J = 4.5$, 10.5)	4.52 (*dd*, $J = 4.5$, 10.7)
H_b–C(1)	4.41 (*dd*, $J = 4.5$, 10.5)	4.43 (*dd*, $J = 5.0$, 10.6)
H–C(2)	5.09–5.13 (*m*)	5.12 (*m*)
H–C(3)	4.34 (*dd*, $J = 4.5$, 6.5)	4.36 (*dd*, $J = 4.6$, 6.6)
H–C(4)	4.26–4.32 (*m*)	4.29 (*m*)
H–C(2′)	4.62 (*dd*, $J = 4.0$, 8.0)	4.63 (*dd*, $J = 4.0$, 7.6)

[a]) Data for (2*S*,3*S*,4*R*)-2-[(2*R*)-2-Hydroxytetracosanoylamino]hexadecane-1,3,4-triol (=(2*R*)-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)pentadecyl]-2-hydroxytetracosanamide) from [20].

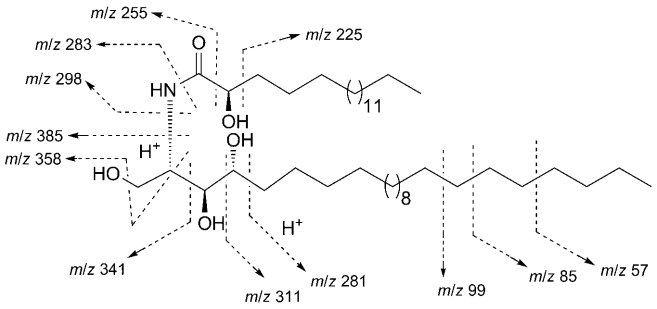

Fig. 2. *Mass fragmentation pattern of citropremide* (**1**)

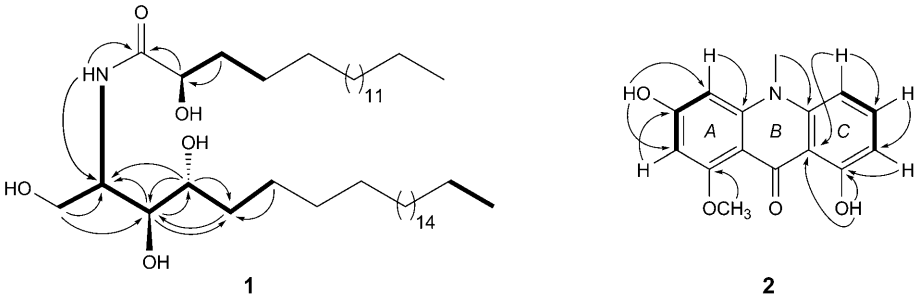

Fig. 3. *Key* $^1H,^1H$*-COSY* (—) *and HMBC* (H → C) *features of citropremide* (**1**) *and citropridone* (**2**)

and 4.34 (H–C(3)). Furthermore, H_a–C(1) showed correlations with H–C(2). No cross-peaks were observed from the signal at δ(H) 4.62 (H–C(2′)) to any downfield H-atom signals but in the HMBC spectrum, it showed a strong correlation to δ(C) 175.7 (C(1′)). This suggested that the fourth OH group is present at C(2′) of the fatty-acid chain. The position of the three OH groups in the long-chain base was further confirmed from the HMBCs (*Fig. 3*) as well as from the mass fragmentation pattern (*Fig. 2*). The length of the fatty acid was determined by the characteristic ions (*Fig. 2*) at *m*/*z* 283 ($[Me(CH_2)_{15}CH(OH)CO]^+$), 300 ($[Me(CH_2)_{15}CH(OH)CONH_2+H]^+$), and 355 ($[Me(CH_2)_{15}CH(OH)C(OH)=NC(=CH_2)CH_2OH]^+$) in the EI-MS. The length of the long-chain base was also determined by the characteristic ions at *m*/*z* 342 ($[M-Me(CH_2)_{19}(CHOH)_2+H]^+$), 341 ($[Me(CH_2)_{19}(CHOH)_2]^+$), and 358 ($[Me(CH_2)_{19}(CHOH)_2OH]^+$) in the EI-MS [5–19]. The assignments were further confirmed by $^1H,^1H$-COSY, HMQC, and HMBC data (*Fig. 3*). Thus, the long-chain base and fatty acid of **1** must be 2-aminotetracosane-1,3,4-triol and 2-hydroxyoctadecanoic acid, respectively. The ^1H-NMR spectrum of **1** corresponded to that of the synthetic ceramide (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]hexadecane-1,3,4-triol, with regard to the signals due to H_a–C(1), H_b–C(1), H–C(2), H–C(3), H–C(4), and H–C(2′). (*Table*) [20]. The above NMR data and comparison of the optical rotation of compound **1** ($[\alpha]_D = +10.09$) and of this synthetic ceramide ($[\alpha]_D = +9.1$) [20] as well as of a related naturally occurring ceramide [21] suggested that

compound **1** has the same absolute configuration for the core structure of the C(2)–C(3)–C(4) and C(2′) part. On the basis of this evidence, the structure of **1** is proposed to be (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxyoctadecanoylamino]tetracosane-1,3,4-triol[1]).

Citropridone (**2**) was obtained as a yellow amorphous powder and showed the molecular-ion peak in the HR-EI-MS at *m*/*z* 271.0838 corresponding to the molecular formula $C_{15}H_{13}NO_4$. The IR spectrum exhibited vibration bands due to OH groups (3420 cm^{-1}) and a conjugated C=O group (1639 cm^{-1}). The UV absorptions at 253, 280, 303, and 345 nm indicated **2** to be a 9-acridone derivative [22]. The ^{1}H-NMR spectrum of **2** (see *Exper. Part*) revealed the presence of a chelated and a free OH function at δ(H) 14.66 (*s*, 1 H) and 8.10 (br. *s*, 1 H), respectively, both of them exchangeable with D_2O. The ^{1}H-NMR spectrum of **2** also showed signals due to *ABC*-type aromatic H-atoms at δ(H) 7.65 (*dd*, $J = 7.6$, 1.2 Hz, H–C(7)), 7.23 (*dd*, $J = 7.6$, 1.2 Hz, H–C(5)), and 7.12 (*t*, $J = 7.6$ Hz, H–C(6)), indicating that ring *C* was a 1,2,3-trisubstituted benzene ring. The ^{1}H-NMR spectrum of **2** also showed two *meta* aromatic H-atoms at δ(H) 6.18 (*d*, $J = 1.2$ Hz, H–C(2)) and 6.51 (*d*, $J = 1.2$ Hz, H–C(4)), one MeN group at δ(H) 3.91 and one MeO group at 3.92, indicating that the aromatic ring *A* was a tetrasubstituted benzene ring. The presence of an Me and one MeO group in compound **2** was supported further by fragment ions at *m*/*z* 256 ($[M - Me]^+$) and 240 ($[M - MeO]^+$) in its EI-MS. The corresponding H- and C-atom assignments were further accomplished by HMBC and COSY experiments as shown in *Fig. 3*. The two phenolic OH groups (δ(H) 14.66 and 8.10) were located at C(8) and C(3), respectively, as determined by the HMBC cross-peaks of the former H-atom with C(8), C(7), and C(8a), and of the latter H-atom with C(3), C(4), and C(2). The two *meta* aromatic H-atoms (δ(H) 6.18 and 6.51) were positioned at C(2) and C(4), respectively, as determined by the HMBC cross-peaks of the former H-atom with C(1), C(3), C(4), and C(9a), and of the latter H-atom with C(2), C(3), and C(4a). The position of the MeO group was confirmed to be C(1) as its H-atoms exhibited a HMBC to C(1). All data (*Fig. 3*) suggested that the MeO and OH group must be present at C(1) and C(3), respectively, of ring *A*, and the OH group at C(8) of ring *C* of **2**. Consequently, **2** was established to be 3,8-dihydroxy-1-methoxy-10-methylacridin-9(10*H*)-one and named citropridone after the producing organism, *Citropsis gabunensis.*

Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; *Merck*, 0.040–0.063 mm), and *Sephadex LH-20* (*Amersham Biosciences*). Anal. and prep. TLC: precoated SiO_2 plates *G60* F_{254} or *G50 UV-254*; *Merck*), resp. Optical rotation: *Perkin-Elmer-241-MC* polarimeter; at the Na_D line. UV/VIS Spectra: *Shimadzu UV-2101PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-510P* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-Avance-500* spectrometer; at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm rel. to residual $CDCl_3$ (δ(H) 7.26; δ(C) 77.0) as internal standard, *J* in Hz. EI- and HR-EI-MS: *MAT-8200* and *Micromass-LCT* mass spectrometers; in *m*/*z* (rel. %).

Plant Material. The stem bark of *C. gabunensis* (ENGL.) SWINGLE & M. KELLERM. (Rutaceae) were collected at Mengomo (45 km of Ebolowa) in South Cameroon, in March 2010, and identified by Mr. *Louis Zapfack* (plant taxonomist), Department of Biology and Plant Physiology, University of Yaounde I, Cameroon. A voucher specimen (No. 28383/SRF/CAM) has been deposited with the National Herbarium, Yaounde, Cameroon.

Extraction and Isolation. Dried and powdered stem bark (6 kg) were extracted with MeOH at r.t. for 48 h. The crude extract was suspended in H_2O and extracted with AcOEt to yield an AcOEt fraction (150 g). The AcOEt fraction was then subjected to CC (SiO_2, hexane, hexane/AcOEt, and AcOEt, in order of increasing polarity): *Fractions 1–5*. *Fr. 4* (with hexane/acetone 5.5 : 4.5) yielded citropremide (**1**; 5.2 mg). *Fr. 3* (with hexane/acetone 4 : 1) was re-subjected to CC: citropridone (**2**; 7.9 mg).

Citropremide (*(=(2*R*)-*N*-[(1*S*,2*S*,3*R*)-2,3-Dihydroxy-1-(hydroxymethyl)tricosyl]-2-hydroxyoctadecanamide*; **1**): Colorless powder. M.p. 139°. $[\alpha]_D^{20} = +10.09$ ($c = 0.92$, $CHCl_3$/MeOH). IR ($CHCl_3$/MeOH): 3610, 2940, 2860, 1640, 1297. ^{1}H-NMR (500 MHz, C_5D_5N): 0.85 (*t*, $J = 6.5$, Me(18′)), Me(24)); 1.30 (*s*, 58 H, CH_2(7) to CH_2(23), CH_2(4′) to CH_2(15′)); 1.70–1.71 (*m*, CH_2(3′)); 1.73–1.75 (*m*, CH_2(6)); 1.86 (*m*, CH_2(5)); 4.26–4.32 (*m*, H–C(4)); 4.34 (*dd*, $J = 4.5$, 6.5, H–C(3)); 4.41 (*dd*, $J = 4.5$, 10.5, H_b–C(1)); 4.51 (*dd*, $J = 4.5$, 10.5, H_a–C(1)); 4.62 (*dd*, $J = 4.0$, 8.0, H–C(2′)); 5.09–5.13 (*m*, H–C(2)); 8.55 (*d*, $J = 8.8$, NH). ^{13}C-NMR (125 MHz, C_5D_5N): 14.7 (Me(18′), Me(24)); 23.4 (C(22)); 26.3 (C(17′), C(23)); 27.1 (C(16′)); 30.1 (C(8) to C(21), C(4′) to C(15′)); 32.6 (C(6)); 33.4 (C(5)); 34.7 (C(3′)); 53.5 (C(2)); 62.5 (C(1)); 73.0 (C(2′)); 73.5 (C(4)); 77.4 (C(3)); 175.7 (C(1′)). EI-MS: *Fig. 2*. HR-EI-MS: 683.6420 (M^+, $C_{42}H_{85}NO_5^+$; calc. 683.6428).

Citropridone (*=3,8-Dihydroxy-1-methoxy-10-methylacridin-9(10*H*)-one*; **2**): Yellow crystals. M.p. 239°. UV ($CHCl_3$): 253 (3.71), 280 (3.10), 303 (3.10), 345 (3.04). IR ($CHCl_3$): 3420, 1639, 1610, 1590, 710. ^{1}H-NMR (500 MHz, $CDCl_3$): 3.91 (*s*, MeN); 3.92 (*s*, MeO); 6.18 (*d*, $J = 1.2$, H–C(2)); 6.51 (*d*, $J = 1.2$, H–C(4)); 7.12 (*t*, $J = 7.6$, H–C(6)); 7.23 (*dd*, $J = 7.6$, 1.2, H–C(5)); 7.65 (*dd*, $J = 7.6$, 1.2, H–C(7)); 8.10 (br. *s*, OH–C(3)); 14.66 (*s*, OH–C(8)). ^{13}C-NMR (125 MHz, $CDCl_3$): 41.5 (MeN); 56.2 (MeO); 90.6 (C(4)); 94.9 (C(2)); 104.9 (C(9a)); 116.3 (C(7)); 120.6 (C(5)); 123.0 (C(6)); 123.6 (C(8a)); 147.6 (C(10a)); 147.8 (C(4a)); 166.2 (C(1)); 164.6 (C(3), (C(8)). EI-MS: 271.1 (30, M^+). HR-EI-MS: 271.0838 (M^+, $C_{15}H_{13}NO_4^+$; calc. 271.0845).

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