Phytochemistry, Vol. 26, No. 9, pp. 2593–2595, 1987.
Printed in Great Britain.

0031-9422/87 \$3.00 + 0.00
Pergamon Journals Ltd.

SESQUITERPENE LACTONES FROM *GUTENBERGIA CORDIFOLIA*

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(*Received* 28 *October* 1986)

Key Word Index—*Gutenbergia cordifolia*; Compositae; sesquiterpene lactones; germacranolide; eudesmanolide; idomain; gutenbergin; stenophyllolide diacetate.

Abstract—*Gutenbergia cordifolia* afforded an eudesmanolide, gutenbergin, and a germacranolide, idomain. The structures of the new sesquiterpene lactones were established by spectral methods, mainly ^{1}H NMR. X-ray analysis of idomain showed that this germacranolide has the common chair-like crown conformation ($[^{15}D_{5,\,1}D^{14}]$conformation) of *trans,trans*-germacra-1(10), 4-dien-*trans*-6,12-olides.

INTRODUCTION

In connection with our investigation of central African plants which are known as folk medicines with antimicrobial and anthelmintic properties we report here the isolation and structure determination of two sesquiterpene lactones from *Gutenbergia cordifolia* Benth. ex Oliver [1] (Compositae, Vernonieae, Vernoniinae), called idoma in Rwanda. The major constituent was a new eudesmanolide (**1**), which we named gutenbergin; a minor one was a new germacranolide (**2**), named idomain. To our knowledge no chemical studies on *Gutenbergia* sp. have been published to date. However, some sesquiterpenes have been isolated from *Erlangea* sp. which may be a genus closely related or identical to *Gutenbergia* sp. [2]

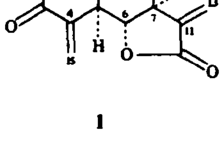
RESULTS AND DISCUSSION

Chloroform extracts of dried and powdered leaves of *G. cordiflora* collected in Rwanda furnished two sesquiterpene lactones in *ca* 0.1 % and 0.01 % yields. The major one, which we named gutenbergin (**1**), $C_{17}H_{18}O_5$ (based on $[M]^+$ at *m/z* 302 and elemental analysis), mp 189–191°, $[\alpha]_D^{25}$+ 87° (MeOH), contained an α-methylene-γ-lactone (1770 cm^{-1}), an α,β-unsaturated ketone (1670 cm^{-1}) and an ester (1735 cm^{-1}) as indicated by the IR bands. The UV absorption (λ_{max} 217 and 239 nm) supported the presence of the former two functionalities. Comparison of the ^{1}H NMR spectrum of **1** with the published data of irazunolide, an eudesmanolide isolated from *Hieracium irazuensis* [3], showed a close similarity, indicating an identical partial structure, i.e. a 1,4(15)-dien-3-one, for ring A. The substitution pattern and stereochemistry of ring B were established by ^{1}H NMR decoupling experiments which connected H-5 through H-9. The large values (11.0–12.6 Hz) of the coupling constants, $J_{5,6}$, $J_{6,7}$, $J_{7,8a}$, and $J_{8a,9}$, indicated a *trans*-diaxial arrangement of these hydrogens. Thus gutenbergin was shown to possess formula **1**. The ^{13}C NMR spectral data of **1** (see Experimental section) were completely in accord with assigned structure.

Table 1. ^{1}H NMR spectral data for guterbergin (**1**) (200 MHz, $CDCl_3$, TMS as internal standard)

Hydrogen	Value
H-1	6.91 *d*
H-2	6.10 *d*
H-5	3.12 *dt*
H-6	4.15 *t*
H-7	2.76 *t br*
H-8a	1.77 *q*
H-8b	2.53 *dt*
H-9	4.97 *dd*
H-13a	5.48 *d*
H-13b	6.18 *d*
H-14	1.15 *s*
H-15a	5.81 *d*
H-15b	6.37 *d*
AcO	2.17 *s*

J (Hz): 1,2 = 10.3; 5,6 = 11.0; 6,7 = 11.0; 7,8a = 12.5; 7,8b = 4; 8a,8b = 12.6; 8a,9 = 11.1; 8b,9 = 4.6; 7,13a = 3.1; 7,13b = 3.2; 5,15a = 1.7; 5,15b = 1.9.



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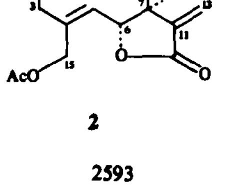

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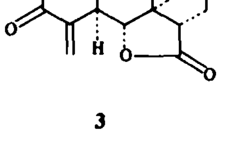

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The absolute stereochemistry of **1** was established as follows. Hydrolysis of **1** with methanolic KOH followed by acidification with 2N HCL afforded a desacetyl compound **3**, whose structure was established on the basis of the ^{1}H NMR and IR spectral data. The configuration at the C-11 position was deduced from the large value (13 Hz) of $J_{7,11}$. Application of the modified Horeau method [4] to alcohol **3** resulted in formation of the (*R*)- and (*S*)-amides in the ratio 100:92. Therefore the C-9 configuration was assigned as *S*, which implies that the absolute stereochemistry of **1** is also represented by formula **1**.

The minor sesquiterpene, idomain (**2**), $C_{19}H_{24}O_6$ (based on $[M]^+$ at *m/z* 348 and elemental analysis), mp 161–162°, $[\alpha]_D^{23}$+ 83° (MeOH), contained an α-methylene-γ-lactone (1760 cm^{-1}) and esters(s) (1730 cm^{-1}) as indicated by the IR bands. The presence of the former functionality was supported by the UV absorption (λ_{max} 223 nm) and the ^{1}H NMR spectrum (exo-methylene signals at δ5.58 and 6.31). The presence of acetate groups was indicated by two methyl signals at δ2.10 and 2.06 in the ^{1}H NMR spectrum. These spectroscopic data, including the ^{1}H NMR data listed in Table 2, were in good agreement with those published for stenophyllolide diacetate, which was chemically derived from stenophyllolide, a germacranolide isolated from *Centaurea aspera* var. *stenophylla* [5]. However, the mp of idomain was considerably different from that reported for stenophyllolide diacetate (> 200°).

In order to ascertain the structure of **2** and to know the conformation of **2**, an X-ray analysis of **2** was carried out, although the X-ray analysis of stenophyllolide was previously reported [6]. Idomain crystallized in the orthorhombic space group $P_{2_12_12_1}$ and possessed unit cell constants of a = 11.3520 Å, b = 7.9220 Å, c = 20.4282 Å with Z = 4. The perspective view of idomain is represented in Fig. 1, which unequivocally confirmed the identity of idomain and stenophyllolide diacetate. It can be seen from Fig. 1 that the cyclodecadiene ring adopts a chair-like crown conformation ($[^{15}D_{5,1}D^{14}]$ conformation) in which both the methyl group (C-14) and acetoxymethyl group (C-15) are β-directed and the C-9-acetoxy group is situated equatorially. This conformation is very similar to stenophyllolide [6] and costunolide [7, 8].

Table 2. ^{1}H NMR spectral data for idomain (**2**) (200 MHz, $CDCl_3$, TMS as internal standard)

Hydrogen	Value
H-1	5.27 *m*
H-2a	*ca* 2.27 *m*
H-2b	*
H-3a	2.58 *m*
H-3b	*
H-5	4.84 *d*
H-6	4.60 *t*
H-7	2.74 *t br*
H-8a	1.95 *dt*
H-8b	2.15 *m*
H-9	5.23 *dd*
H-13a	5.58 *d*
H-13b	6.31 *d*
H-14	1.43 *s br*
H-15	4.60 *s*
AcO	2.06 *s*
AcO	2.10 *s*

*Multiplet in the region δ2.05–2.25.

J (Hz): 1,2a = 5.4; 1,2b = 9.7; 5,6 = 10.0; 6,7 = 9.3; 7,8a = 10.9; 8a,8b = 14.3; 8a,9 = 10.9; 8b, 9 = 2.9; 7, 13a = 3.0; 7,13b = 3.3.

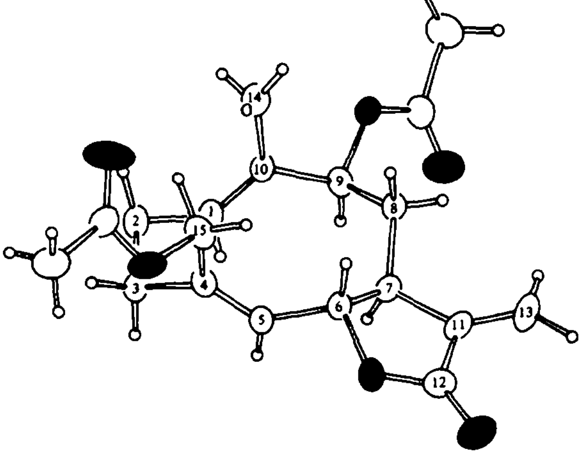

Fig. 1. Perspective view of idomain.

The following argument on the absolute configuration of idomain can be given. The strongly positive Cotton effect at 217 nm due to the interaction between the double bonds in the cyclodecadiene ring is in accord with those observed for costunolide and other *trans*, *trans*-germacra-1(10),4-dien-*trans*-6,12-olides of established absolute configuration, i.e. with H-7α [9, 10]. Further, the negative Cotton effect at 258 nm due to the α-methylene-γ-lactone chromophore *n*-π* transition state is in accord with the Stocklin rule [10]. Thus it is very probable that idomain belongs to the series of 7α-H-germacranolide as specified in formula **2**.

It appears that the presence of a 9β-acetoxy group is one of the features of the sesquiterpenes of this plant. Gutenbergin exhibited antibacterial activity and cytotoxicity.

EXPERIMENTAL

Isolation of **1** *and* **2**. Dried and ground leaves (500 g) of *Gutenbergia cordifolia* collected in Oct. 1984 near Butare in Rwanda (Voucher No. J-M-4a which is deposited at Department of Chemistry, Tokyo Institute of Technology) was extracted with $CHCl_3$ (2 × 3 l) producing *ca* 50 g of a dark residue. The residue was chromatographed over silica gel (900 g) eluting with $CHCl_3$ containing increasing proportions of EtOAc. The fraction eluted with $CHCl_3$–EtOAc (4:1) was rechromatographed similarly. The $CHCl_3$–EtOAc (6:1) fraction (2 g) was then chromatographed on Sephadex LH-20 (2 cm × 70 cm) eluting with MeOH. The fractions, which showed UV absorption, were combined (0.9 g) (careful TLC analysis of the fraction indicated the presence of two compounds with very close R_f values) and purified on silica gel column eluting with hexane–EtOAc (2:1). The sesquiterpene mixture eluted (*ca* 0.6 g) was crystallized from MeOH to afforded 200 mg of **1** as the first crops, mp 189–191°, $[\alpha]_D^{25}$ + 87° (MeOH; *c* 0.23), UV λ_{max}^{MeOH} nm: 217 (ε 6600) and 239 (ε 8200); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1735, 1670, 1620 and 970; CD spectrum

(MeOH): 253 nm, $\Delta\varepsilon + 4.8$; 238 nm, $\Delta\varepsilon + 0$; 226 nm, $\Delta\varepsilon - 2.6$; MS *m/z*: 302 $[M]^+$, 260 $[M - CH_2CO]^+$, 242 $[M - MeCOOH]^+$ and 43 $[MeCO]^+$ (base peak). ^{13}C NMR $\delta^{\mathrm{TMS}}_{\mathrm{CDCl_3}}$: 15.23 (*q*), 21.00 (*q*), 26.34 (*t*), 42.88 (*s*), 46.24 (*d*), 51.04 (*d*), 74.09 (*d*), 77.88 (*d*), 118.11 (*t*), 123.39 (*t*), 128.12 (*d*), 136.87 (*s*), 139.06 (*s*), 153.23 (*d*), 169.25 (*s*), 170.15 (*s*), 186.66 (*s*). (Found: C, 67.50; H, 6.10. $C_{17}H_{18}O_5$ requires: C, 67.54; H, 6.00%).

One-third of the mother liquid of the above crystallization was separated by prep. TLC (Merck silica gel 60 F_{254} precoated plates, 20 cm × 20 cm, developed with hexane–EtOAc 3:1, several times). From the less polar UV-absorbing band, 15 mg of **2** was obtained, after recrystallization from MeOH, mp 161–162°, $[\alpha]^{25}_{D} + 83°$ (MeOH; *c* 0.13), UV $\lambda^{\mathrm{MeOH}}_{\mathrm{max}}$ nm: 223 (ε5100); IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1}: 1760, 1730, 970; CD spectrum (MeOH): 258 nm, $\Delta\varepsilon - 2.1$; 244 nm, $\Delta\varepsilon$ 0; 217 nm, $\Delta\varepsilon + 31$; MS *m/z*: 348 $[M]^+$, 289 $[M - MeCOO]^+$, 247 $[M - MeCOO - CH_2CO]^+$, 229 $[M - MeCOO - MeCOOH]^+$, 43 $[MeCO]^+$ (base peak). ^{13}C NMR $\delta^{\mathrm{TMS}}_{\mathrm{CDCl_3}}$: 11.55 (*q*), 20.92 (*q*), 21.30 (*q*), 26.08 (*t*), 33.31 (*t*), 35.44 (*t*), 47.37 (*d*), 61.43 (*t*), 77.62 (*d*), 79.40 (*d*), 120.59 (*t*), 130.68 (*d*), 130.68 (*d*), 135.15 (*s*), 138.12 (*s*), 138.44 (*s*), 169.16 (*s*), 169.77 (*s*), 170.47 (*s*). (Found: C, 65.58; H, 6.99. $C_{19}H_{24}O_6$ requires: C, 65.50; H, 6.94%).

Determination of the absolute stereochemistry of **1**. To compound **1** (50 mg) dissolved in 2ml MeOH–THF (1:1) was added 5% KOH in MeOH (1 ml). The mixture was stirred at room temp. for 10 hr and acidified by the addition of 2N HCl (2 ml). The whole was allowed to stir for 30 min and extracted with EtOAc. The organic layer was washed with NaCl soln, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was applied on a prep. TLC plate (doubly developed with hexane–EtOAc, 2:1). The strongest UV-absorbing band (R_f 0.4) afforded compound **3** (20 mg) as a solid: mp 208–210°; IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1}: 1775, 1670, 1620; ^{1}H NMR $\delta^{\mathrm{TMS}}_{\mathrm{CDCl_3}}$: 1.07 (3H, *s*, 10-Me), 1.73 (1H, *q*, $J = 11.5$ Hz, 9-H), 1.83 (1H, *d*, $J = 6.0$ Hz, 9-OH), 2.25 (2H, *m*, 8-H and 7-H), 2.63 (1H, *ddd*, $J = 13, 5.6, 4.0$ Hz, 11-H), 2.94 (1H, *dt*, $J = 10.3, 2.4$ Hz, 5-H), 3.38 (3H, *s*, OMe), 3.66 (1H, *dd*, $J = 10.5, 5.6$ Hz, 13-H), 3.75 (1H, *dd*, $J = 10.5, 4$ Hz, 13-H'), 3.81 (1H, *m*, 9-H), 4.15 (1H, *t*, $J = 11.0$ Hz, 6-H), 5.71 (1H, *d*, $J = 2$ Hz, 15-H), 6.12 (1H, *d*, $J = 10.2$ Hz, 3-H), 6.32 (1H, *d*, $J = 2$ Hz, 15-H'), 7.31 (1H, *d*, $J = 10.2$ Hz, 1-H). Compound **3** (1.0 mg) was treated with (±)-α-phenylbutyric anhydride (2 μl) and pyridine (18 μl) at 40° for 2 hr. (±)-(*R*)-α-Phenylethylamine (2 μl) was added and the mixture was allowed to stand for 15 min with occasional shaking, and analysed, after dilution with EtOAc, by GC (1% OV-17, 1 m × 4 mm i.d. glass column, oven temp. 200°, injection temp. 230°). The ratio of the peak area at 5.8 min (*R*-amide) and 6.6 min (*S*-amide) was compared to that of the control run for cyclohexanol.

X-ray analysis of **2**. X-ray analysis was performed on a four-circle diffractometer by using CuKα radiation ($\lambda = 1.54178$ Å). A single crystal of **2** was obtained by crystallization from MeOH. A total of 1961 independent reflections were measured for $2\theta < 156°$. The structure was solved by direct methods with MULTAN80 [11]. In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic were used for the hydrogen atoms. The final discrepancy index are $R = 0.053$ for the observed reflections.

Acknowledgements—The authors thank Mr Shi-Pin Hong for assistance of extraction and purification of the compounds. Thanks are also due to the National Herbarium, Butare, Rwanda, for the identification of the plant and Prof. Akiko Itai, Tokyo University for the X-ray analysis.

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