Isagarin, a New Type of Tetracyclic Naphthoquinone from the Roots of *Pentas longiflora*

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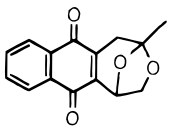
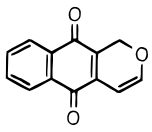
A new type of tetracyclic naphthoquinone, named isagarin (**1**), was isolated from the hexane extract of the roots of *Pentas longiflora*. On the basis of spectral and X-ray diffraction analysis this compound was identified as 1,4-epoxy-4-methyl-1,2,4,5-tetrahydronaphtho[2.3-d]oxepin-6,11-dione.

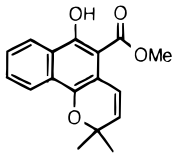
Pentas longiflora Oliv. (Rubiaceae) is an erect-stemmed woody herb up to 3 m high from oriental intertropical Africa,[1] which is reputed to possess several medicinal properties.[2] In Rwanda, where it is known in the traditional medicine under the name "Isagara", the powder of the roots, mixed with butter, is used as an ointment to treat scabies[3] and the skin mycosis *Pityriasis versicolor*.[4] Some attention has been devoted to the constituents of *P. longiflora*; pentalongin (**2**) and mollugin (**3**), two naphthoquinones, were isolated from the root bark.[5] In a bioassay-guided phytochemical investigation of the roots, the active principle responsible for the antifungal activity was isolated and identified as pentalongin, and an antimycotic ointment was developed with the EtOH extract of the roots of *P. longiflora*.[6] The interesting biological activity of *P. longiflora* led to a detailed phytochemical study of this plant in order to identify the minor compounds. In the present report, the isolation and structure determination of isagarin (**1**), a new type of tetracyclic naphthoquinone from the roots of *P. longiflora*, is described.



1 (Isagarin)



2 (Pentalongin)



3 (Mollugin)

The hexane extract of the dried roots of *P. longiflora* was fractionated by MPLC on Si gel and further purified by centrifugal partition chromatography (CPC) to afford isagarin (**1**) as yellow crystals. Isagarin (**1**) was identified on the basis of its spectral properties. A molecular ion at *m*/*z* 256 is compatible with the molecular composition of $C_{15}H_{12}O_4$ (see below). The ^{1}H NMR spectrum ($CDCl_3$) with two two-proton multiplets centered at δ 7.73 (Ar−H) and δ 8.08 (Ar−H) and the IR spectrum with absorptions at 1596 cm^{-1} (C═C) and 1632, 1661 cm^{-1} (C═O) indicated the presence of a naphthoquinone moiety. The ^{1}H NMR also showed the presence of a singlet signal of a methyl group (δ 1.68, 3H) and a methylene group (δ 2.73, d, 2.87, d × d), the latter showing a geminal coupling constant of 19.6 Hz. The one proton signal at δ 5.58, showing a doublet with J = 4.0 Hz, is indicative for a CH−O unit substituted at the carbon adjacent to one of the carbonyl groups of the naphthoquinone nucleus. This oxygenated methine group holds a position adjacent to a CH_2O unit, which shows a degenerate spin system at δ 3.93−4.05. The 2D COSY spectrum showed the right connectivities of the aromatic hydrogens, indicative of an unsubstituted aromatic ring of a 1,4-naphthoquinone unit. In addition, this spectrum underlined the direct coupling of a CH−O unit with a CH_2−O unit, and the presence of an isolated methylene function, substituted onto the benzoquinone skeleton. The ^{13}C NMR spectrum ($CDCl_3$) revealed the presence of two oxygenated carbons (CH_2O and CHO), a very typical acetal signal at δ 106.36 and a 1,4−naphthoquinone skeleton as major features. All signals of the DEPT spectrum and the HETCOR spectrum could be easily assembled to the previously assigned units.

When all structural units are put together, compound **1** (isagarin) was identified as 1,4-epoxy-4-methyl-1,2,4,5-tetrahydronaphtho[2.3-d]oxepin-6,11-dione. Apparently, this is a novel skeleton encountered in naturally occurring naphthoquinones. The mass spectrum shows the molecular ion (M^+; 28%) and the very typical *m*/*z* 43 fragment at largest abundance. Although an acetyl group is not present in isagarin (**1**), the formation of this fragment ion can be explained by cleavage of the C4−C5 bond, which generates a stable oxonium ion **4** with a pendant allyl radical. Subsequent homolytic cleavage of the oxygenated oxonium ion (see **5**) and splitting off the acetoxonium ion explains this peculiar fragmentation (Scheme 1).

Because of the fact that isagarin **1** concerned a novel skeleton in natural products, its structure was verified and secured by X-ray crystallographic analysis (Figure 1).

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a JEOL EX 270 NMR spectrometer (270 MHz for ^{1}H NMR, 68 MHz for ^{13}C NMR). IR spectra were obtained using a Perkin−Elmer 1310 spectrophotometer. MS were recorded on a Varian MAT 112 mass spectrometer (70 eV) using the direct inlet system.

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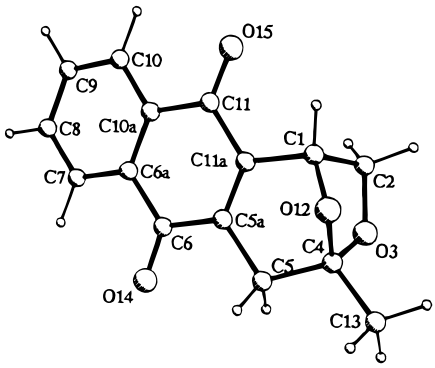
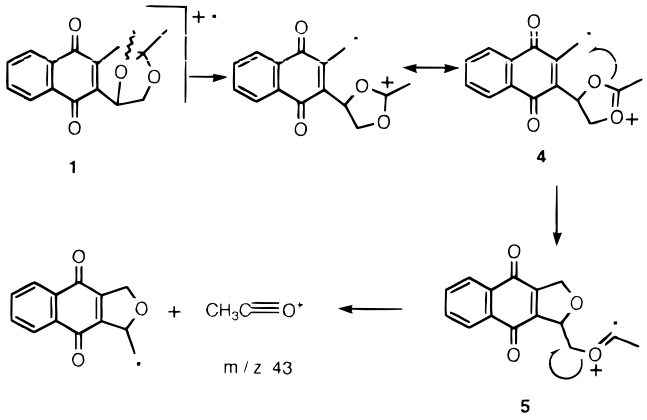


Figure 1. X-ray crystallographic picture of isagarin (**1**).

Scheme 1



Plant Material. Roots of *P. longiflora* were collected in the prefecture of Butare (southwestern Rwanda) in May 1991. The plant was identified by one of the authors (L. Van Puyvelde), and a voucher herbarium species (LVP no. 374) was deposited in the herbarium of the Institut de Recherche Scientifique et Technologique (I. R. S. T.), Butare, Rwanda. The roots were dried in an oven at 40 °C and powdered mechanically.

Extraction and Isolation. The powder of the roots (4100 g) was extracted in a percolator with hexane (5 × 5000 mL) to give after evaporation, a dark red extract (44.0 g). This hexane extract was submitted to MPLC on Si gel (Merck, Si gel 60, 0.015−0.040 mm) and eluted with mixtures of hexane−EtOAc of increasing polarity. Fractions eluted with hexane−EtOAc (75:25) (1710 mg) were further fractionated by CPC model CCC-1000, Pharma-Tech Research Corp.; solvent system, hexane−EtOAc−MeOH−H_2O 9:1:5:5; reversed phase; flow rate, 1.0 mL/min; rotor speed, 1000 rpm; detection at 254 and 280 nm; 5 runs), which gave 10 fractions. Fraction 6 (181 mg; 0.004%) yielded compound **1** upon evaporation of the solvent in vacuo.

Isagarin (1): yellow parallelepiped crystals, mp 160.9−161.4° (from MeOH); $[\alpha]_D$ −12° (*c* 0.25, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 1661 (C=O), 1632 (C=O), 1596 (C=C), 1396, 1328, 1297 1215, 1165, 1092, 1076, 1049, 1010; ^1H NMR (270 MHz, $CDCl_3$) δ 1.68 (3H, s, Me), 2.73 (1H, d, J = 19.6 Hz, C5−H), 2.87 (1H, d × d, J = 19.6, 1.32 Hz, C5−H), 3.93−4.05 (2H, m, CH_2O), 5.58 (1H, d, J = 4.0 Hz, C1−H), 7.70−7.78 (2H, m, C8−H and C9−H), 8.04−8.13 (2H, m, C7−H and C10−H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 24.00 (Me), 36.32 (C-5), 70.17 (C-1), 73.01 (C-2), 106.36 (C-4), 126.32 and 126.43 (C-7 and C-10), 133.87 and 133.92 (C-8 and C-9), 131.62, 132.07, 141.72, and 144.00 (C-5a, C-6a, C-10a and C-11a), 182.58 and 184.17 (C-6 and C-11); EIMS *m/z* (rel int) 256 (28; M^+), 226 (43), 214 (20), 213 (11), 197 (69), 196 (52), 186 (18), 185 (14), 169 (9), 158 (9), 157 (11), 141 (11), 139 (7), 129 (7), 128 (9), 127 (9), 115 (12), 105 (15), 104 (7), 77 (12), 76 (16), 69 (9), 57 (7), 55 (4), 43 (100).

X-ray Structure Analysis of 1. $C_{15}H_{12}O_4$, M_r = 256.3, orthorhombic, space group $P2_12_12_1$, unit cell dimensions: a = 5.191 (3) Å, b = 7.710 (4) Å, c = 29.894 (13) Å; V = 1196 (1) $Å^3$; Z = 4; D_x = 1.42 g·cm^{-3}, Cu Kα, λ = 1.5418 Å, μ = 8.72 cm^{-1}, $F(000)$ = 536, temperature (K) 291; R = 0.054 for 2103 observed reflections. D_m was not measured. Parallelepiped crystal with dimensions 0.03 × 0.1 × 0.4 mm was used. Lattice parameters were refined using 16 reflections in the range 20° ≤ 2θ ≤ 40°. A Huber four-circle diffractometer was used with a graphite monochromatized Cu Kα radiation; 3015 measured reflections were with sin $\theta/\lambda \leq 0.53$ $Å^{-1}$; $-5 \leq h \leq 5$, $0 \leq k \leq 8$, $0 \leq l \leq 31$; 1509 independent reflections (R-merge = 0.048), and 1173 with $I \geq 2.5\sigma(I)$. A standard reflection (−1−1 8) was checked every 50 reflections with no significant deviation. The structure was solved by direct methods using SHELXS-86.[7] H atoms are in computed positions. Anisotropic least squares refinement (SHELX-76[8]) was applied using F; H isotopic with common refined temperature factor (U = 0.10 $Å^2$); $w = 1/(\sigma^2 + 0.00917F^2)$, R = 0.054, wR = 0.059, S = 0.77 for 1173 observed reflections. A final maximum shift to error = 0.04 was obtained. The maximal and minimal heights in final difference Fourier synthesis were 0.22 and − 0.38 e·$Å^{-3}$. Atomic scattering factors were taken from the International Tables for X-ray Crystallography. The atomic parameters, bond distances and angles, and torsion angles of compound **1** are deposited as Supporting Information in the Cambridge Crystallographic Data Centre.[9]

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(9) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-(0)1223−336033, or E-mail: deposit@ccdc.cam.ac.uk).

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