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CHEMISTRY OF *SAURURUS CERNUUS*, V.[1] SAURISTOLACTAM AND OTHER NITROGENOUS CONSTITUENTS

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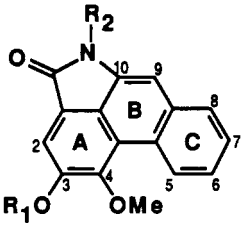
ABSTRACT.—Two aristololactam analogues were isolated from the extract of *Saururus cernuus*. One of these was the known aristololactam BII [**1**] (cepharanone B) and the other new, named sauristolactam [**2**], was shown to be the lactam of 10-aminomethyl-3-hydroxy-4-methoxyphenanthrene-1-carboxylic acid. Here we detail the first reported occurrence of compounds of the aristololactam group in the genus *Saururus*.

Saururus cernuus L. (Sauruaceae) is an aquatic weed from which two novel dineolignans named manassantins A and B have been isolated earlier and shown to exhibit significant neuroleptic activity (1,2). A series of other neolignans also have been isolated which represent a variety of structural types (3). This note deals with the isolation of two isomeric, nonlignoid, fluorescent compounds, designated initially as FA and FB, and their characterization.

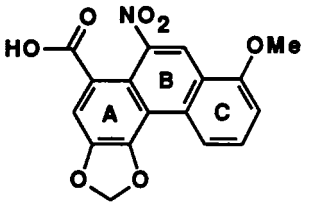
Partition of the concentrated EtOH extract of the above-ground parts of the plant between H_2O and $CHCl_3$ transferred both FA and FB, which appeared as bright fluorescent spots in tlc, into the organic layer, together with all of the lignoid and other lipid-soluble components. Separation into the neutral and phenolic components by base extraction gave FA in the neutral fraction and FB in the phenolic fraction. Final purification was effected by Si gel chromatography and crystallization.

FA, $C_{17}H_{13}NO_3$, was identified as aristololactam BII (cepharanone B) [**1**] (4) based on spectral data (see Experimental). FB, $C_{17}H_{13}NO_3$, was phenolic, and its uv spectrum resembled closely that of FA, except for the bathochromic shift induced by base. The prominent 1690 cm^{-1} peak in the ir spectrum of **2** indicated the presence of a lactam ring similar to that seen in the spectrum of FA. The ^{1}H-nmr spectrum showed signals at δ 3.32 (3H) and 4.10 (3H) indicative of an NMe and an OMe group, respectively. On the assumption that FB also possessed an aristololactam skeleton, the nmr spectrum showed further that, apart from two singlet protons at δ 7.60 and 7.16, there were four other aromatic protons whose complex coupling patterns when resolved by decoupling gave the following results. Irradiation of the signal attributable to H-5 collapsed the signals due to H-6 and H-7 from td to dd. Likewise, irradiation of the H-6 and H-7 signals changed the H-5 and H-8 signals from dd to singlets. These results showed that the complex pattern was due to four vicinal aromatic protons. There was an exchangeable proton signal at δ 10.26, attributable to the phenolic OH. The chemical shifts of the OH and OMe precluded the possibility that either of these groups was present peri to the lactam carbonyl. These two groups are, therefore, located at C-4 and C-3. This substitution pattern in ring A is in accordance with that seen in FA and in all other related aristololactams (4–6). The absence of a bathochromic shift in the uv spectrum of FB when treated with NaOAc suggested that the OH was at C-3 and OMe at C-4. In support of this, it was found that methylation of the OH to **3** or acetylation to form **4** caused a significant deshielding of the C-2 proton, the signal moving from 7.6 to 7.78 in **3** and to 7.80 in **4**. ^{13}C-nmr spectral evidence confirmed the structure as **2**.

[1]For Part IV, see S.K. Chattopadhay and K.V. Rao, *Tetrahedron*, **43**, 669 (1987).



1 R_1=Me, R_2=H
2 R_1=H, R_2=Me
3 R_1=R_2=Me
4 R_1=Ac, R_2=Me



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Finally, methylation of both **1** and **2** gave the identical product **3**. FB is named sauristolactam [**2**].

It was possible that compound **2** might be an artifact derived from a 4:5-dioxoaporphine type precursor which under basic conditions might undergo a benzylic acid rearrangement to yield **2** (7). Because the isolation of **2** did involve extraction into base, it would be important to establish this point. In support of the natural occurrence of **2**, it is possible to see both **1** and **2** in the tlc of the initial $CHCl_3$ extract as fluorescent spots, although because their R_f values are very close, multiple development was necessary, with the other and somewhat major components of the extract being also present in this region. Also, the conditions of base extraction (0.1 N NaOH, 23° and 30 min) used for the isolation of **2** are not generally sufficient to produce the benzylic acid rearrangement (8). However, in spite of these observations, an unequivocal proof would be necessary and is provided here.

Although **1** and **2** have somewhat close R_f values, acetylation converts **2** to **4** which is readily separable from either **1** or **2**. Accordingly, the initial $CHCl_3$ extract (obtained without the use of any base) was first subjected to Si gel chromatography and the fraction containing the fluorescent spot(s) separated and acetylated. The product was purified by preparative tlc. Of the two fluorescent spots, the faster moving [R_f 0.6, C_6H_6-Me_2CO (9:1)] was found to be identical with a reference sample of **4**, and when the R_f 0.6 sample was hydrolyzed with methanolic acid, the product was identical with **2** by spectral and chromatographic comparison. These results clearly establish that **2** was naturally occurring in the plant.

The term aristololactam is commonly used to designate compounds derivable in the laboratory from aristolochic acid [**5**] by reduction of the nitro group followed by lactamization and containing the structural unit dibenzo[*cd,f*]indol-4(5*H*)one with oxygen substituents at various positions, e.g., 3, 4, 6, 8, or 9. Some members are being given other names such as enterocarpam A/B, cepharanone, taliscamine, and in the present case sauristolactam, to avoid the confusing terminology that exists in the literature such as aristololactam AIII, III, IIIa, and AIIIa to designate four different compounds; it is easy to confuse one name with the other (5,6). To add further confusion, those members which have a methylenedioxy group at C-3, C-4 are listed in *Chemical Abstracts* under a different structural unit: benzo[*f*]-1,3-benzdioxolo[6,5,4-*cd*]-indol-5(6*H*)-one. None of the members reported so far have been shown to contain the NMe function seen with sauristolactam [**2**]. Isolation of these two compounds records the occurrence of the aristololactams in the genus *Saururus* for the first time.

From the aqueous layers of the extract remaining after the $CHCl_3$ extractions have been completed, a colorless crystalline solid with phenolic properties was also isolated. Its molecular formula, $C_9H_{11}NO_3$, the strongly positive ninhydrin reaction, and direct comparison with an authentic sample showed that the compound was L-tyrosine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on Fisher-Johns apparatus and are uncorrected. The following instruments were used to record the spectra described here: uv, Perkin-Elmer Lambda 3B, with MeOH as solvent; ir, Beckman, Aculab III as KBr pellets; nmr, Varian EM 390, 90 MHz instrument, $CDCl_3$ with TMS as internal standard; optical rotations, Perkin-Elmer 141 polarimeter, 1% in $CHCl_3$; and eims, Kratos MS 80 RFA. Cc was performed using Si gel (Merck 100–200 mesh) and tlc with Si gel (Merck H60-P254/366).

PLANT MATERIAL.—Above-ground parts of *S. cernuus* were collected during May–July 1987, in Gainesville, Florida. A voucher sample was deposited at the Herbarium, University of Florida, #FLAS 170066. The material was sun-dried and ground to a course mesh.

EXTRACTION.—The ground plant (10 kg) was extracted with MeOH at 20° for 3 days. The extract and two such subsequent extracts were concentrated to a syrup (1 liter) and extracted twice with an equal volume of $CHCl_3$. The solvent extract was concentrated to a glass, taken up in C_6H_6 (1 liter), and extracted three times with 20% aqueous MeOH containing NaOH (0.1 N). The organic layer was washed with H_2O and concentrated to a small volume to give the neutral fraction (125 g). The alkaline layers were acidified and extracted with $CHCl_3$, and the extract was concentrated to a glass to give the phenolic fraction (10 g).

ISOLATION OF **1**.—The neutral fraction in 25-g portions was taken up in C_6H_6 (200 ml) and the solution applied to a column of Si gel (250 g). The eluent was incrementally changed to reach 10% Me_2CO in C_6H_6, and 100-ml fractions were collected throughout. Fractions from 2–4% Me_2CO which contained **1** were concentrated to dryness and taken up in Et_2O (25 ml). The insoluble solid was filtered and crystallized from $CHCl_3$-C_6H_6 (3:1) to yield pale yellow needles (0.62 g): mp 262–264° [lit. (4) 263–265°]; uv max (log ε) 230 (4.41), 261 (4.27), 275 (4.35), 285 (4.33), 314 (3.78), 380 (3.74) nm; ir 3180, 1710, 1370, 1105, 730 cm^{-1}; ^1H nmr (DMSO-d_6) δ 4.05, 4.08 (2s, 6H, 2OMe), 7.16 (s, 1H, H-9), 7.88 (s, 1H, H-2), 7.59 (td, J = 7.0 and 2.5 Hz, 2H, H-6 and H-7), 7.98 (dd, J = 7.0 and 2.5 Hz, H-8), 9.13 (dd, J = 7.0 and 2.5 Hz, H-5), 10.89 (s, NH); ^{13}C nmr 56.90, 59.90, 104.6, 109.9, 119.9, 121.5, 123.3, 125.4, 125.9, 126.8, 127.4, 129.0, 134.8, 135.1, 150.4, 154.2, 168.4; ms *m/z* 279 (100), 264 (10), 221 (12), 193 (19), 163.

ISOLATION OF **2**.—The phenolic fraction was dissolved in C_6H_6 (50 ml), and the solution was applied to a column of Si gel (60 g) as described earlier. The fractions that contained **2** were combined and concentrated to dryness, and the residue was crystallized first from Et_2O and later from $CHCl_3$-hexane (3:1) to yield **2** as pale yellow prisms (0.36 g): mp >290°; uv 235 (4.56), 262 (4.42), 276 (4.46), 287 (4.45), 314 (3.94), 388 (3.88) in base, 258 (4.72), 290 (4.49), 296 (4.57), 310 (4.35), 346 (4.13), 425 (4.03) nm; ir 3445, 1690, 1640, 1420, 1310, 1250, 960, 730 cm^{-1}; ^1H nmr (DMSO-d_6), 3.32 (s, 3H, NMe), 4.0 (s, 3H, OMe), 7.16 (s, 1H, H-9), 7.60 (s, 1H, H-2), 7.53 (td, J = 7.0 and 2.5 Hz, H-6 and H-7), 7.86 (dd, J = 7.0 and 2.50 Hz, 1H, H-8), 9.09 (dd, J = 7.0 and 2.5 Hz, H-5), 10.26 (s, 1H, OH); ^{13}C nmr 26.04 (NMe), 59.45 (OMe), 103.3, 113.5, 120.1, 121.0, 125.4, 126.3, 126.8, 127.3, 128.9, 134.6, 136.8, 148.7, 152.1, 166.7; ms *m/z* 279 (100), 264 (52), 236 (28), 180 (19), $[M]^+$ 279.0890 ± 0.0020. Calcd for $C_{17}H_{13}NO_3$, 279.0895.

METHYLATION OF **2** TO **3**.—A solution of **2** (0.03 g) in Me_2CO (10 ml) was boiled under reflux with Me_2SO_4 (0.05 ml) and anhydrous K_2CO_3 (0.5 g) for 3 h. The mixture was concentrated to dryness and diluted with H_2O, and the solid was filtered and crystallized from $CHCl_3$-MeOH (3:2): mp 193–194°; uv 230 (4.63), 260 (4.49), 275 (4.51), 286 (4.46), 316 (3.97), 382 (3.93) nm; ir 2940, 1690, 1640, 1450, 1400, 1310, 1230, 1110, 1010, 950, 740 cm^{-1}; ^1H nmr ($CDCl_3$) 3.46 (s, 3H, NMe), 4.05, 4.10 (2s, 6H, 2OMe), 6.94 (s, 1H, H-9), 7.78 (s, 1H, H-2), 7.55 (td, J = 7.0, 2.5 Hz, H-6 and H-7), 7.85 (dd, J = 7.0, 2.5 Hz, 1H, H-8), 9.18 (dd, J = 7.0, 2.5 Hz, H-5). *Anal.* calcd for $C_{18}H_{15}NO_3$, C 73.70, H 5.15, N 4.78; found C 73.62, H 5.08, N 4.72.

ACETYLATION OF **2** TO **4**.—A mixture of **2** (0.03 g), Ac_2O (0.2 ml), and pyridine (0.1 ml) was heated at 100° for 30 min. After cooling and addition of H_2O (10 ml), the solid was filtered and crystallized from C_6H_6-ligroin (2:1): mp 243–245°; uv 250 (4.46), 260 (sh) (4.41), 276 (4.25), 285 (4.31), 376 (3.71) nm; ^1H nmr ($CDCl_3$) 2.43 (s, 3H, Ac), 3.42 (s, 3H, NMe), 4.03 (s, 3H, OMe), 6.96 (s, 1H, H-9), 7.50 (td, J = 7.0, 2.5 Hz, 2H, H-6 and H-7), 7.75 (dd, J = 7.0, 2.5 Hz, 1H, H-8), 7.80 (s, 1H, H-2), 9.0 (dd, J = 7.0, 2.5 Hz, 1H, H-5). *Anal.* calcd for $C_{19}H_{15}NO_4$, C 71.02, H 4.71, N 4.36; found C 71.11, H 4.75, N 4.28.

METHYLATION OF **1**.—Methylation of **1** was carried out as described for compound **3**. The product after crystallization was identical with **3** by mp, mmp, uv, ir, nmr spectra, and tlc.

PROOF OF NATURAL OCCURRENCE OF **2**.—An aliquot of the original MeOH extract concentrate containing approximately 10 mg of **1** and **2** was partitioned between H_2O (50 ml) and $CHCl_3$ (50 ml). The organic layer was concentrated to an oil, dissolved in C_6H_6 (10 ml), and applied to a column of Si gel (25 g) in C_6H_6. Elution with 2–5% Me_2CO in C_6H_6 gave the bulk of the two fluorescent compounds in the eluate. The fractions were combined, concentrated, and heated with Ac_2O (2 ml) and pyridine (0.4 ml) at 100° for 5 min. After addition of H_2O, extraction with $CHCl_3$, and washing of the organic layer successively with dilute acid, aqueous bicarbonate, and H_2O, the $CHCl_3$ was evaporated to dryness. The residue was dissolved in C_6H_6 (1 ml) and applied to a preparative tlc plate from 30 g of Si gel, and the plate was developed in 10% Me_2CO in C_6H_6. The compounds from the two fluorescent bands were recovered and compared with the reference samples by spectral and chromatographic properties. The compound with R_f 0.6 was identical with **4** and the one with R_f 0.3 with **1**.

L-TYROSINE.—The residual aqueous layer from the $CHCl_3$ extractions of the original concentrate (2 liters) was freed from $CHCl_3$, and a 200-ml portion was passed through a column of Amberlite XAD-2 (38 × 300 mm) to absorb the glycosidic pigments. The effluent and the aqueous wash were concentrated to 20 ml and set aside. The solid which crystallized was filtered, washed with H_2O, and recrystallized from HOAc: yield 0.25 g; mp >290°; $[\alpha]_D$ −9° (1%, 1 N HCL). Direct comparison with an authentic sample showed that the compound was L-tyrosine.

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