Naphthoquinones and Anthocyanins from two *Drosera* Species

GERD BENDZ and GÖSTA LINDBERG

Institute of Chemistry, University of Uppsala, Box 531, 751 21 Uppsala 1, Sweden

Drosera intermedia has been found to contain two anthocyanins and several naphthoquinones. The same pigments were also isolated from *D. anglica*.

By preparative thin layer chromatography four naphthoquinones were isolated. The most abundant was identified as plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) which has earlier been isolated from *D. intermedia* and from other *Drosera* species.[1,2]

The remaining naphthoquinones were isolated in so small amounts that only their absorbance in the 200–600 nm region and their mass spectra could be recorded. By comparing these spectral data with those of an authentic specimen and by co-chromatography one naphthoquinone was identified as 2-methylnaphthazarin (5,8-dihydroxy-2-methyl-1,4-naphthoquinone). Another quinone exihibited a molecular ion base peak at $m/e=222$ with a satellite peak at $m/e=224$. The relative intensities of M+2 to M as well as those of the M−CO peak ($m/e=194$) to its satellite peak ($m/e=196$) were 36 % which suggested the presence of a chlorinated quinone. Peaks at $m/e=120$, 92, and 63 indicated the presence of a hydroxyl group in the benzenoid ring.[3] By comparing the spectral and chromatographic properties of this quinone with an authentic specimen it was identified as 3-chloroplumbagin (3-chloro-5-hydroxy-2-methyl-1,4-naphthoquinone). Since this is the first chlorinated naphthoquinone found in a phanerogam its occurrence was rather unexpected.

A fourth quinone isolated in too small amount for further purification exhibited spectral properties rather similar to 7-methyljuglone (5-hydroxy-7-methyl-1,4-naphthoquinone). When more material is available this quinone as well as the possible existence of a fifth quinone will be more fully studied.

The two anthocyanins isolated from each *Drosera* species were proved from spectral and chromatographic data to be cyanidin and pelargonidin glycosides.

Experimental. The absorption spectra were recorded on a Spectronic 505 spectrophotometer. The molecular weights and the mass spectra were determined with an LKB 9000 mass spectrometer.

Preparative TLC was performed on Merck's Silica Gel G and co-chromatography on Eastman "Chromatogram sheets" type K 301. The solvent systems used were: benzene-chloroform (2:3 v/v), cyclohexane-chloroform (7:3 v/v), benzene-cyclohexane (3:2 v/v), petroleum ether-ethyl acetate (7:3 v/v) and hexane-chloroform (2:1 v/v). The identities of the isolated quinones from the two *Drosera* species with the synthetic specimens were proved by co-chromatography in these five solvent systems and by mass spectrometry.

D. intermedia was collected at Siggeforasjön about 20 km NW of Uppsala and *D. anglica* in the parish of Idre in northwestern Dalecarlia.

The dried plant material was extracted with ether containing 1 % conc. H_2SO_4. After filtration, evaporation, and addition of 10 ml 2 M H_2SO_4 the residue was steam distilled. The steam volatile naphthoquinones were extracted with ether and separated by repeated preparative TLC in benzene-chloroform (2:3 v/v).

Band 1 (R_F: 0.80–0.85) gave after elution a yellow compound. M=222. λ_{max} (ethanol): 216, 249, 280 and 424 nm. The spectral data were identical with those of a synthetic sample of 3-chloroplumbagin, m.p. 125–126°.[4]

Band 2 (R_F: 0.7–0.8) yielded after elution and recrystallization orange-yellow needles, M=188, m.p. 75.5–77.5°.[5] The spectral data were identical with those reported for plumbagin.[3,6]

Band 3 (R_F: 0.45–0.50) contained a very small amount of a yellow pigment, M=188. The mass spectrum was similar to that of 7-methyljuglone.

Band 4 (R_F: 0.35–0.45) gave after elution a red-brown compound, M=204. λ_{max} (ethanol): 216, 278, 486, 514, 552 nm. The spectral data were identical with those of synthetic 2-methylnaphthazarin, m.p. 174–175° [7]).

The anthocyanins were isolated from the plant material and further purified according to methods described earlier.[8] The aglycons were identified as cyanidin and pelargonidin by co-chromatography in the usual solvents and from their ultraviolet and visible absorption spectra.

Acknowledgements. The authors wish to express their gratitude to Professor Arne Fredga who collected the material used and put the necessary facilities at their disposal.

A grant from the *Royal Swedish Academy of Science* (Kemiska prisgruppens särskilda fond) is gratefully acknowledged.

1. Karrer, W. *Konstitution und Vorkommen der organischen Pflanzenstoffe*, Basel 1958, p. 487.
2. Paris, R. and Delaveau, P. *Ann. Pharm. France* **17** (1959) 585.
3. Bowie, J. H., Cameron, D. W. and Williams, D. H. *J. Am. Chem. Soc.* **87** (1965) 5094.
4. Thomson, R. H. *J. Chem. Soc.* **1949** 1277.
5. Fieser, L. F. and Dunn, J. T. *J. Am. Chem. Soc.* **58** (1936) 572.
6. Cooke, R. G. and Dowd, H. *Australian J. Sci. Res.* **A 5** (1952) 760.
7. Farina, F., Lora-Tamayo, M. and Suarez, C. *Tetrahedron Letters* **1959** 9.
8. Bendz, G. and Haglund, Å. *Acta Chem. Scand.* **21** (1967) 2286.

Received September 16, 1968.

Structure and Function of Sphingolipids

2. Differences in Sphingolipid Concentration, Especially Concerning Sulfatides, between Some Regions of Bovine Kidney

KARL-ANDERS KARLSSON, BO E. SAMUELSSON and GÖRAN O. STEEN

Institute of Medical Biochemistry, University of Göteborg, Göteborg, Sweden

In an attempt to correlate sphingolipid structure with tissue function, kidney was chosen as an object of study, as almost all known animal sphingolipids have been detected in this organ. The first paper in this series [1] reports qualitative as well as quantitative differences in the total long-chain base fractions from different regions of kidney. As a development of this, different classes of intact sphingolipids have been prepared from the same regions.

Fresh bovine kidneys were macroscopically dissected into cortex, medulla, and papillae. To obtain pure preparations of cortex and medulla, the transition zone was collected separately. Only small apices were taken as papillae in this dissection. From another number of kidneys, the apices were cut somewhat larger.

The homogenized and lyophilized tissue was extracted in hot chloroform-methanol (2/1, v/v), once with 20 ml/g dry tissue, and four times with 10 ml/g. This procedure was found to leave less than 2 % of the total long-chain bases, measured gravimetrically on dinitrophenyl (DNP) base fractions,[1] isolated after acid hydrolysis of remaining substance. The combined extracts were evaporated to dryness and submitted to alkaline degradation for 24 h at 37°C, using 1 M KOH in water, 10 ml/g lipid. After acidification to pH 2 with HCl, partition was done (chloroform-methanol-water 8:4:3, v/v/v). The upper phase from a total kidney preparation contained only 0.4 % of the total long-chain bases, measured as DNP derivatives, why the lower, lipid phase was taken alone for the preparations.

Fractionation by column chromatography was performed principally according to Rouser *et al.*[2], using silicic acid, magnesium silicate (Florisil) and diethylaminoethyl (DEAE) cellulose. Purity of fractions was tested by thin-layer chromatography. For identification, references of human brain and kidney sphingolipids were used, as well as infrared spectroscopy and analysis of hydrolysis products (carbohydrates,[3] long-chain bases,[1] and fatty acids). The figures given in Table 1 were determined gravimetrically.

The most prominent difference found concerns the sulfatides, which have a far higher concentration in medulla than in cortex. In medulla, the papillary part has a somewhat lower concentration. The total neutral glycolipids as well as the purified cerebrosides increase in concentration from cortex to papillae. On the other hand, the sphingomyelins, the dominating fraction, decrease in concentration from cortex to papillae. The composition of the transition zone is generally intermediary, compared to those of cortex and medulla. Cerebrosides are the dominating glycolipids, in contrast to human kidney cerebrosides, which are small components (0.22 mg/g dry tissue).[4] Tetraglycosyl ceramides are only trace components in bovine kidney, while they are the main glycolipids in human kidney (1.77 mg/g dry tissue).[4] Sulfatides with two hexose residues [5] have not been