Acospectoside A, A New Cardenolide Glycoside

Sir:

In an examination of triterpenoid constituents of the leaves of *Acokanthera spectabilis* (Sond.) Hook. f., isolation and characterization of friedelin was reported (1, 2). Subsequently, stem triterpenes were studied, and friedelin was also isolated from the petroleum ether extract of this plant part. Besides friedelin, another crystalline compound, $C_{30}H_{50}O$, was obtained from stem petroleum ether extract. This second compound was characterized as lupeol by elemental analysis, mass spectrum determination, comparison of I.R. and NMR spectra, R_f values on thin-layer chromatograms, and mixed melting point of the isolated and authentic sample.

A study of cardenolide glycosides of leaves and stems of *A. spectabilis* was also performed. To isolate these compounds from the leaves, the dried powdered plant material,[1] previously deprived of petroleum ether extractives, was extracted with chloroform and methylene chloride. The combined residues from chloroform and methylene chloride extracts were extracted with petroleum ether, dissolved in 70% aqueous ethanol, and shaken in succession with petroleum ether and mixture of benzene–petroleum ether (70:30). The aqueous alcoholic layer was purified by treatment with lead hydroxide according to Reichstein *et al.* (3), then filtered and concentrated to remove alcohol. The aqueous concentrate was extracted with chloroform. The chloroform extract (A) and the remaining aqueous layer (B) were separately evaporated to dryness.

Paper chromatographic examination of extracts A and B [using Whatman No. 1 paper containing ~35% water and developing with toluene–*n*-butanol (4:1)–water] revealed the presence of eight cardenolide glycosides in B, five of which were present in A.

Crystallization of residue from A with ethanol furnished a crystalline product (C), m.p. 192–195° (softens) and 291–293°. Chromatography of the product (C) on paper indicated that it contained a new cardenolide glycoside, designated acospectoside A, as a major constituent, and a small amount (5–10%) of acobioside A,[2] traces of acovenoside A, and another unidentified glycoside.

Pure acospectoside A could be isolated from residue A by column chromatography on neutral alumina or preparative paper chromatography of product (C). The new cardenolide glycoside was obtained as a white crystalline compound, m.p. 290–298°, $[\alpha]_{589}^{25°} = -97°$, $[\alpha]_{436}^{25°} = -147°$, and $[\alpha]_{330}^{25°} = -231°$ (C = 0.078, MeOH); $\lambda_{max.}^{MeOH}$ 217 mμ (E, 1%, 1 cm., 209.8), and $\nu_{max.}^{KBr}$ 3430 (broad), 1745, 1724, 1256, 1240 cm.$^{-1}$.

Anal.—Calcd. for $C_{36}H_{56}O_{14}$: C, 60.65; H, 7.92. Calcd. for $C_{38}H_{58}O_{15}$: C, 60.46; H, 7.74. 1%, 1 cm. Found: C, 60.67, 60.71; H, 7.97, 7.87.

Isolation of acobioside A was accomplished by preparative paper chromatography of product (C). The cardenolide glycoside was identified by melting point, enzymatic cleavage with strophanthobiase to acovenoside A (4), and identical I.R. spectra and R_f values on paper and thin-layer chromatograms as that of the authentic sample.

Acovenoside A was isolated from the mother liquor of product (C) and identified by comparison with an authentic sample (melting point, R_f values on paper and thin-layer chromatograms and I.R. spectra). In the fraction from which acovenoside A was isolated, a spot at the acovenoside B R_f location was revealed by paper and thin-layer chromatography.

Acospectoside A, acobioside A, and acovenoside A were also isolated from the stem fractions of *A. spectabilis*. Although acobioside A has been prepared by enzymatic splitting of glucose from acovenoside C (4), *A. spectabilis* appears to be the first plant from which it is isolated in a crystalline form and identified.[3]

Preliminary toxicity assay of acospectoside A was performed in cats and the glycoside was found

[1] The leaves and stems used in this study were collected from the U. S. Botanic Garden, Washington, D. C., and soon thereafter dried in a Stokes electrically heated forced air-circulation tray-type drying oven at a temperature not exceeding 60°.

[2] That the acospectoside A was a new glycoside was confirmed by Professor T. Reichstein, University of Basel, who also indicated that the product (C) contained a small amount (5–10%) of acobioside A which according to him and Dr. Ek. Weiss could be separated from the new compound on a paper standardized to contain 35% water based on the dry weight of the paper and using toluene–*n*-butanol (4:1)–water for development of chromatogram.

[3] In a personal communication Professor Reichstein informed that P. Hauschild (Dissertation, University of Basel, 1963) had separated glycoside fractions of *A. oppositifolia* seeds. According to him, Hauschild's glycoside 9 (not isolated in pure form) had identical running properties on paper chromatograms as acospectoside A and his glycoside 10 (also not isolated in pure form) had R_f values identical with acobioside A.

to be active. However, the potency was not very high. In three cats the geometric mean lethal dose was found to be 1.806 mg./Kg.

Acospectoside A and acobioside A were also tested on isolated perfused guinea pig heart. Both the glycosides caused a positive ionotropic action. Results of preliminary testing suggested that in case of acospectoside A, increase in contractility was rapid in onset but brief in duration, and was approximately proportional to the dose. In a guinea pig heart, doses of 2 and 25 mcg. caused 110 and 167% increase of contraction, respectively.

Effect of acobioside A was comparatively slow in onset but persistent in duration. In the same heart it appeared to be 3–10 times more potent than acospectoside A.

(1) Kapadia, G. J., Kapadia, G. G., and Mosby, J. R., *Lloydia*, **27**, 233(1964).
(2) *Ibid.*, **27**, 272(1964).
(3) Euw, J. V., *et al.*, *Helv. Chim. Acta*, **34**, 1821(1951).
(4) Hauschild-Rogat, P., *et al.*, *ibid.*, **45**, 2116(1962).

GOVIND J. KAPADIA

Department of Pharmacognosy and Natural Products
College of Pharmacy
Howard University
Washington, D. C.

Received September 3, 1965.
Accepted for publication October 6, 1965.

This investigation was supported by grant HE-06571 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

This report is part of a paper presented to the American Society of Pharmacognosy, Pittsburgh meeting, June 1964. (See *Reference 2* for the published abstract.)

The author appreciates the kind cooperation of Professor T. Reichstein, University of Basel, and thanks him for furnishing samples of acovenosides A and B and acobioside A. The author also thanks Professor K. K. Chen, Indiana University, for toxicity assay of acospectoside A on cats; Professor S. N. Pradhan, Howard University, for tests on isolated guinea pig heart; and Dr. R. J. Highet and Mrs. K. S. Warren, National Heart Institute, for spectral data and optical rotation determinations.

Errata

In the article titled "Solubilization of Weakly Acidic and Basic Drugs by Aqueous Solutions of Polysorbate 80" (1), the sentence beginning on line 6, column 2, page 1348, should read:

The greater apparent energy released by partitioning of the ionized acid may be due, in part, to entropy contributions related to the ion–dipole interaction with water which does not occur in the case of the free acid.

(1) Rippie, E. G., Lamb, D. J., and Romig, P. W., *J. Pharm. Sci.*, **53**, 1346(1964).

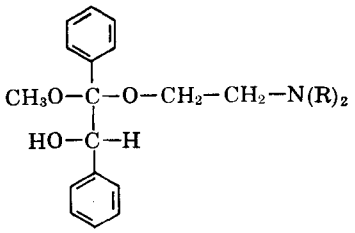
In the article titled "Biopharmaceutical Investigation of Nalidixic Acid in Man" (1), the following corrections should be made:

1. Under the section *In Vitro Dissolution Rates* on page 38, sentence 4 should read: The method consisted of using a phosphate buffer at pH 7.5 in a volume of 400 ml./500 mg. of nalidixic acid at 37°.
2. In Table III on page 40 under "... k_E Min.$^{-1}$ (Range)" for "Micropulverized powder—Caplet" change 0.00019 to 0.0019.
3. In Table IV on page 40 under "Min. Peak Time" for "Sodium salt capsule" change 46 to 48.

(1) Moore, W. E., *et al.*, *J. Pharm. Sci.*, **54**, 36(1965).

In the article titled "Synthesis and Antitremorine Activity of Amino Ketals" (1), the following corrections should be made:

1. On page 60, structure VI should be:



VI

2. On page 61, in Table II under "Compd.," change VI^a to VI*a*.
3. Page 63, column 2, line 3 should read: **1,2-Diphenyl-2-methoxy-2-(β-piperidinoethoxy)-ethanol Oxalate (VIc).—**

(1) Johnson, H. L., and Oneto, J. F., *J. Pharm. Sci.*, **54**, 59(1965).