as above and led in 82% yield to the alcohol Xa. Recrystallization from methanol–chloroform gave the analytical sample with m.p. 236–237° (Kofler), $[\alpha]^{20}D$ −81.3°. The substance showed no selective absorption in the ultraviolet and exhibited no carbonyl bands in the infrared.[9] It was recovered unchanged on treatment with hydrazine hydrate.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77. Found: C, 78.92; H, 9.66.

The acetate Xb, m.p. 260–262° (Kofler), $[\alpha]^{20}D$ −70.7°, after recrystallization from chloroform—methanol, showed no hydroxyl band in the infrared.[9]

Anal. Calcd. for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found: C, 76.33; H, 9.01.

LAGUNA MAYRAN 413
MEXICO CITY 17, D. F.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF S. B. PENICK & COMPANY]

The Glycosides of the Seeds of *Strophanthus intermedius* Pax.

BY M. R. SALMON, ERIC SMITH AND W. G. BYWATER

The seeds of *Strophanthus intermedius* were found to contain two crystalline cardioactive glycosides that appear to be identical with those previously isolated by v. Euw and Reichstein[1] from *Strophanthus gerrardii* and provisionally called Substance 761 and Substance 762. We find that Substance 761 is a glycoside of sarverogenin.[2]

The Upjohn–Penick Expedition[3] for African Botanical Exploration in 1949–1950 collected a large series of the seeds of *Strophanthus* species for study of their glycoside content in an effort to make a complete survey of the availability of glycosides containing 11-oxygenated steroid nuclei suitable for conversion to cortisone or related steroids. The elegant work of Reichstein and his group has outlined the general method for isolation of glycosides from *Strophanthus* species, details of which have been described.[1,2,4] One species of *Strophanthus* which has not previously been studied in detail has now been subjected to the general isolation technique developed in Professor Reichstein's laboratories. This species was identified as *Strophanthus intermedius* (B&W No. 20932) by Mr. Joseph Monachino of the New York Botanical Garden. The seeds were collected in northwestern Angola.

The seeds were worked up according to the general procedure of Katz.[4a] The resulting aqueous solution of the total glycosides was extracted successively with ether and with chloroform. The glycosides remaining in the aqueous phase after extraction have not yet been examined.

The ether extract on concentration gave 350 mg. of crystalline material which agreed in properties with Reichstein's Substance 761 except for a slightly higher rotation. In addition there was 389 mg. of oily glycosides that did not yield crystalline material after chromatographic adsorption.

The chloroform extract yielded 6.25 g. of total glycosides which, when crystallized from methanol, gave 3.0 g. of white crystals. The properties of the crystalline material did not change significantly on recrystallization but an attempt to prepare the aglycone from a small portion showed conclusively that it was a mixture of an easily hydrolyzable and a difficultly hydrolyzable glycoside. Accordingly, the crystalline mixture was chromatogramed and there was isolated 636 mg. of Reichstein's Substance 761 and 392 mg. of Reichstein's Substance 762, and other crystalline fractions which may have been mixtures of these two.

The oily mother liquors from the 3 g. of crystalline glycosides were separately chromatogramed and we found some chromatogram fractions with a small negative rotation, suggesting the presence of sarmentocymarin, but we were unable to isolate this glycoside.

The total yield of ether- and chloroform-soluble components from *Strophanthus intermedius* seeds was 4.5%, of which 2.2% was crystalline glycosides.

The preparation identified as Reichstein's Substance 761 on hydrolysis with dilute acid gave sarverogenin, characterized as the crystalline benzoate. Since Reichstein's Substance 761 appears to be isomeric with sarveroside, the sugar portion of the molecule is probably isomeric with sarmentose. We have not examined the sugar formed on hydrolysis.

The occurrence of Reichstein's Substance 761 in the seeds of *Strophanthus intermedius* has been announced in a footnote (reference 1, page 525). The simultaneous occurrence of Reichstein's Substance 762 and the characterization of Substance 761 as a glycoside of sarverogenin have not previously been reported. In addition to *Strophanthus gerrardii* and *S. intermedius*, Substance 761 and Substance 762 have been found in *S. courmonti*[4b] and Substance 762 has been found in *S. sarmentosus*.[4g]

We are indebted to Professor T. Reichstein who has examined our two crystalline glycosides and reported to us that the colors formed with 84% sulfuric acid are identical with the corresponding glycosides prepared in his laboratory and there is no depression in melting point when corresponding

(1) J. v. Euw and T. Reichstein, *Helv. Chim. Acta*, **33**, 522 (1950).

(2) A. Buzas, J. v. Euw and T. Reichstein, *ibid.*, **33**, 465 (1950).

(3) An expedition sponsored jointly by The Upjohn Company of Kalamazoo, Michigan, and S. B. Penick & Company of New York, of which Mr. L. J. Brass of the Archbold Expeditions unit of The American Museum of Natural History was field director and Mr. E. F. Woodward of S. B. Penick & Company was pharmacognosist and business manager. Herbarium specimens of all the materials collected by the expedition have been submitted to the New York Botanical Garden for taxonomic study. Such specimens have been assigned field numbers under the name of Brass and Woodward; for example, one of the *Strophanthus intermedius* samples collected by the expedition is identified as B&W No. 20932. A set of the collection will also be on deposit with the Royal Botanic Gardens, Kew, Surrey, England. When the taxonomic studies are completed it will be possible to identify and locate the species of *Strophanthus*, upon which chemical isolation work is being done in these laboratories, by reference to the appropriate numbers in the collection.

(4) For example (a) A. Katz, *Helv. Chim. Acta*, **31**, 993 (1948); (b) J. v. Euw and T. Reichstein, *ibid.*, **31**, 883 (1948); (c) J. v. Euw and T. Reichstein, *ibid.*, **33**, 544, 666 (1950); (d) A. Lardon, *ibid.*, **33**, 639 (1950); (e) John W. Rothrock, E. E. Howe, Klaus Florey and Max Tishler, THIS JOURNAL, **72**, 3827 (1950); (f) J. v. Euw and T. Reichstein, *Helv. chim. acta*, **33**, 1006 (1950); (g) **33**, 2153 (1950).

pairs are mixed, although this is not to be regarded as conclusive evidence of identity.

Experimental

All rotations are in methanol at 1–1.5% concentration unless otherwise noted. All melting points were taken with a hot stage microscope and are uncorrected. Keller–Kiliani tests were run in homogeneous solution.[4b] Sulfuric acid color tests were run with 84% acid in thin layers on watch glasses, under which conditions the change in colors is more rapid than when the test is carried out on porcelain spot plates. Preparations for analysis were dried at 120–130° (0.2 mm.) for four hours.

The light greenish-tan seeds had 5.95% moisture and 30.6% fat. Slices of seeds treated with 84% sulfuric acid became tan (1 min.) light brown (3 min.) purplish-brown (5 min.) and gray-blue (10–30 min.).

The seeds (150 g., mostly immature) were coarsely ground and exhausted with petroleum ether by percolation. They were dried, remilled and moistened with 150 cc. of water. Toluene (10 cc.) was worked into the paste and it was allowed to autolyze for 72 hours in the 35° incubator. Alcohol (150 cc.) was added and the mixture digested four hours and the extract pressed out in a laboratory press.

The marc was re-extracted with 150 cc. of 80% alcohol for 3–4 hours at 50–60° and again pressed. The extraction was repeated (10 times) until the extracts no longer gave the Keller–Kiliani test for 2-desoxy sugars.

The combined extracts were treated with basic lead acetate in slight excess and the lead precipitate removed by filtration and washed with 80% alcohol. The filtrate was deleaded with hydrogen sulfide, filtered, and concentrated in vacuum to 120–150 cc.

Ether Extract.—The solution was extracted with six 100-cc. portions of ether, the extracts were washed with water, sodium carbonate solution and water, and dried and concentrated. Three hundred and fifty mg. of crystals separated from the concentrated ether solution and after recrystallization from methanol and ether and then from methanol and water had $[\alpha]_D$ +24° (MeOH) and melted at 192–199°. The Keller–Kiliani test was positive. The sulfuric acid color test was identical with that described later for Reichstein's Substance 761 and there was no melting point depression when the two preparations were mixed.

The mother liquor was taken up in a little chloroform and precipitated with ten volumes of petroleum ether. The flocculent precipitate (389 mg.) was chromatogramed on alumina but no further crystalline material was obtained.

Chloroform Extract.—The aqueous solution remaining from the treatment with ether was extracted with five 100-cc. portions of chloroform, the extracts were washed with water, sodium carbonate solution and water, and dried and concentrated. There was 7.3 g. of residual glycosides which crystallized without further treatment. The glycoside mixture was digested with 15 cc. of methanol, filtered and washed with methanol. White crystals (2.1 g., $[\alpha]_D$ +24°, m.p. 208–210°) were obtained. The mother liquors were evaporated to dryness, dissolved in a little chloroform and precipitated with ten volumes of petroleum ether. The flocculent precipitate (4.15 g.) crystallized from acetone and ether and gave an additional 0.9 g. of crystalline material ($[\alpha]_D$ +26.7°, m.p. 202–208°).

The two crops of crystals were combined and unsuccessful attempts were made to isolate homogeneous preparations by crystallization from various solvents. Finally, all crystalline preparations were combined and subjected to chromatographic adsorption.

The crystalline material was chromatogramed on specially prepared[5] alumina and the combined mother liquors were chromatogramed separately. Since the two experiments gave similar results corresponding fractions from the two chromatograms were combined and rechromatogramed on a third column of alumina. The third chromatogram is described in detail in Table I.

Reichstein's Substance 761.—Fractions C, D, E, F and G were rubbed up with a small amount of methanol and filtered and washed with methanol. The total yield of crystalline material was 636 mg. and the samples melted in the range of 176–194° and had rotations of $[\alpha]_D$ +18° to +22°. After recrystallization from aqueous methanol and from acetone and ether, the melting point was 190–193° and the rotation was $[\alpha]_D$ +21.5°. The Keller–Kiliani test was positive. With 84% sulfuric acid the color was brown developing a reddish tinge in 2 min. and a green rim in 5 min., and becoming blue-green and then blue in 20 minutes. The ultraviolet adsorption spectrum showed a plateau in the region 270–280 mμ, log ε 1.9, which is believed to be due to the presence of a carbonyl group.[1,2]

Anal. Calcd. for $C_{30}H_{44}O_{10}$: C, 63.81; H, 7.85. Found: C, 63.51, 63.35; H, 7.59, 7.54.

von Euw and Reichstein[1] report for Substance 761, m.p. 178–180°, $[\alpha]_D$ +18.9°. Keller–Kiliani test positive, carbonyl absorption, sulfuric acid color test and analyses in agreement with those above.

TABLE I

50 g. alumina, 100 cc. of solvent each fraction; fractions from previous chromatogram added with successive portions of solvent; B = benzene, C = chloroform, M = methanol

Fraction	Solvent, %				Yield, mg.	M.p., °C.	$[\alpha]_D$	Keller–Kiliani
A			100	B	1	.....	.....	
B	90	B	10	C	1.5	.....	.....	
C	80	B	20	C	49.5	169–191	+14.7°	+
D	70	B	30	C	424.1	189–192	+13.9	++
E	60	B	40	C	312.8	160–193	+11.6	++
F	50	B	50	C	109.6	179–193	+11.8	++
G	40	B	60	C	56.2	180–193	+12.7	++
H	30	B	70	C	121.4	135–203	+15.1	+
	20	B	80	C				
I	10	B	90	C	218.3	200–233	+20.7	−
J			100	C	182.3	215–231	+16.0	−
K	99	C	1	M	236.7	171–223	+10.7	−
L	95	C	5	M	60.9	.....	+18.2	±
M	90	C	10	M	99.6	.....	+13.5	+

Reichstein's Substance 762.—Fractions I, J and K from the above chromatogram were rubbed up with a little methanol, filtered and washed with methanol. The yield of crystalline material was 392 mg., m.p. 217–232°, $[\alpha]_D$ +26°. After recrystallization from aqueous methanol, from acetone, and from methanol and ether the melting point was 215–226°, $[\alpha]_D$ +29.6°. The Keller–Kiliani test was negative. With 84% sulfuric acid the color was red becoming lilac and developing a blue rim in 2–3 min., and becoming blue which persisted from 5–20 minutes. The ultraviolet absorption spectrum showed carbonyl absorption (270–280 mμ, log ε 1.85). When mixed with Substance 761 there was no melting point depression (m.p. 195–215°).

Anal. Calcd. for $C_{30}H_{44}O_{11}$: C, 62.06; H, 7.64. Found: C, 62.43, 62.19; H, 7.32, 7.05.

von Euw and Reichstein[1] report for Substance 762, m.p. 216–218°, $[\alpha]_D$ +31.7°, Keller–Kiliani test negative, carbonyl absorption, sulfuric acid color test and analyses in agreement with those above.

Sarverogenin from Substance 761.—Substance 761 (50 mg.) was suspended in 0.5 cc. of methanol and 0.05 cc. of water and 0.05 cc. of concd. hydrochloric acid was added. After standing overnight at room temperature the preparation was diluted with 2 cc. of water and seeded, and it then crystallized rapidly. The crystals were filtered and washed with aqueous methanol when they melted at 200–210°, $[\alpha]_D$ +42.3°, yield 25.4 mg.

After recrystallization from methanol and ether and from aqueous methanol, the melting point was 216–223° $[\alpha]_D$ +50.5°. The sulfuric acid color test was identical with that of sarverogenin from sarveroside and there was no melting point depression when the two were mixed.

Anal. Calcd. for $C_{23}H_{32}O_7$: C, 65.67; H, 7.67. Found: C, 66.37, 66.46; H, 7.25, 7.16.

Buzas, v. Euw and Reichstein[2] report for sarverogenin melting point 223–225°, $[\alpha]_D$ + 44.7°, sulfuric acid color test in agreement with our findings, and analyses in agreement with the calculated values.

A second 50-mg. sample of Substance 761 was hydrolyzed

(5) Baker and Adamson reagent aluminum oxide was washed with dilute hydrochloric acid and with water, and air dried. It was extracted continuously for 24 hours with a mixture of chloroform and methanol, dried and activated by heating at 150° for 18 hours. For most purposes 1 g. of glycoside can be chromatogramed on 50 g. of prepared alumina.

as before and the crude aglycone treated with pyridine and benzoyl chloride. The dibenzoate was worked up as described by Buzas, v. Euw and Reichstein and chromatogramed on 2.5 g. of prepared alumina. From the fractions eluted with benzene containing 10 to 40% chloroform there was obtained 31.4 mg. of a benzoate that crystallized from methanol (in which it is very insoluble) and then melted at 223–224°. It was taken up in a little methanol and chloroform, filtered, and the solvent evaporated until crystallization started. Ether was added to complete the crystallization. The melting point was 178–180°, with no double melting point as reported by Buzas, *et al.*, and $[\alpha]D$ +32.0° (acetone). The sulfuric acid color test was very weak: colorless turning pink (1 min.), blue (5 min.) and blue-green (30 min.).

Anal. Calcd. for $C_{37}H_{40}O_9$: C, 70.68; H, 6.41. Found: C, 70.47, 70.52; H, 6.55, 6.42.

Buzas, *et al.*, report for sarverogenin dibenzoate a double melting point 178–184° → 192°, $[\alpha]D$ +31.5°, sulfuric acid color test red becoming violet and developing a blue rim and finally dark blue.

Sarveroside was hydrolyzed to sarverogenin and converted to sarverogenin benzoate as described above. The melting point was 178–182°, $[\alpha]D$ +30° (acetone).

The two samples of sarverogenin benzoate from sarveroside and from Substance 761 were mixed and finely ground in a mortar. The melting point behavior of this mixture was observed on the same cover slip with finely ground portions of the two samples of sarverogenin benzoate and all melted in the same way at the same temperature. When finely ground, sarverogenin benzoate began to melt at 170°, then resolidified and melted at 228–234°.

Acknowledgment.—We wish to thank Dr. Robert W. Price for advice and suggestions, Mr. Louis Pucci for technical assistance, and The Upjohn Company for encouragement and support given during this investigation. Micro-analyses were done by the Schwarzkopf Microanalytical Laboratories.

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Synthesis of Lyxoflavin

BY DOROTHEA HEYL, EDITH C. CHASE, FRANK KONIUSZY AND KARL FOLKERS

L-Lyxoflavin has been synthesized by a route which is different from the one previously described. Calcium D-galacturonate is reduced to calcium L-galactonate, and this product is oxidized to L-lyxose. 3,4-Xylidine and L-lyxose are reductively coupled to form N-L-lyxityl-4,5-dimethylaniline, which is converted to N-L-lyxityl-2-phenylazo-4,5-dimethylaniline. Reaction of the latter compound with barbituric acid yields L-lyxoflavin.

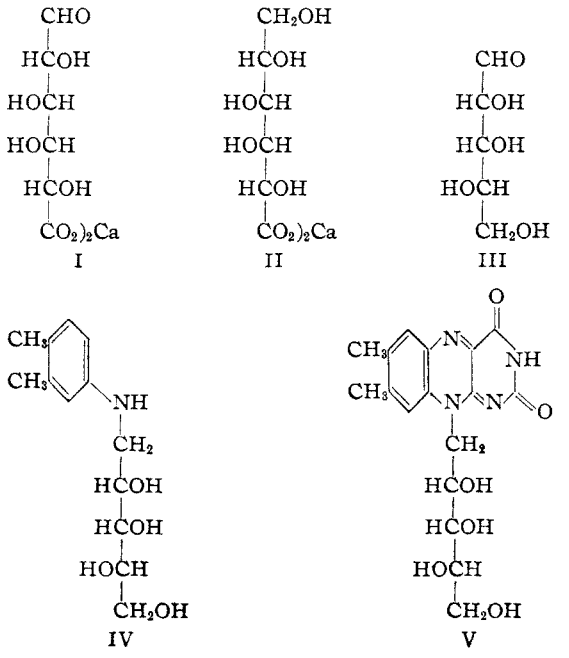
Synthetic L-lyxoflavin has shown growth-promoting or vitamin-like activity[1] in a rat assay for unidentified vitamins in liver and other source materials.

The isolation of lyxoflavin from human myocardium has been described.[2] It was shown that the "natural pentose-flavin" had properties which were identical with those of synthetic L-lyxoflavin. The method[2] of synthesis had previously been used for the synthesis of D-lyxoflavin.[3] This method involved the condensation of 2-carbethoxyamino-4,5-dimethylaniline with D- and L-lyxose to form 1-N-D- and 1-N-L-lyxityl-2-carbethoxyamino-4,5-dimethylaniline. Saponification of the urethans, and condensation of the resulting diamines with alloxan gave D- and L-lyxoflavin.

We have synthesized L-lyxoflavin by a different method. A satisfactory synthesis of L-lyxose combined with an advantageous procedure for the preparation of the flavin, permits lyxoflavin to be made available in sufficient quantities for adequate biological study. D-Galacturonic acid, which is commercially available from citrus pectin, is the source of L-lyxose.

Calcium D-galacturonate (I) was hydrogenated over a Raney nickel catalyst to give calcium L-galactonate (II). Sirupy L-lyxose (III) was prepared by oxidation[4] of calcium L-galactonate with hydrogen peroxide and ferric acetate.

L-Lyxose may be obtained in crystalline form after purification of the crude product by passage through ion exchange columns.[5] The use of pure lyxose for reductive condensation with 3,4-xylidine results in a nearly quantitative yield of N-L-lyxityl-4,5-dimethylaniline (IV); however, the crude sirupy lyxose may be used for the reductive condensation, and the yield of the xylidine derivative (IV) appears to represent the purity of the crude L-lyxose. N-L-Lyxityl-4,5-dimethylaniline coupled readily with diazotized aniline to form N-L-lyxityl-2-phenylazo-4,5-dimethylaniline, and this compound was converted to L-lyxoflavin (V) by direct condensation with barbituric acid.



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(2) Pallares and Garza, *Arch. Biochem.*, **22**, 63 (1949).
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