The ultraviolet absorption spectra in chloroform show principal maxima at 257.5, 327.5, 363.5 and 380.5 mμ, ε 47,800, 9,600, 9,510 and 9,710, respectively. The infrared spectra in Nujol mull in the 6–8 μ region show bands at 6.28, 6.42, 6.55, 6.75, 7.06, 7.32, 7.52, 7.57, 7.97 and 8.05 μ.

3-Bromotropolone (III).—3-Bromotropolone hydrobromide (11.00 g.) from bromination of I, was stirred with 100 ml. of water and 200 ml. of ether until all the solid was dissolved. The layers were separated and the aqueous layer was washed with 50 ml. of ether. The combined ether solutions were dried over magnesium sulfate. Evaporation *in vacuo* to dryness gave 7.90 g. (100%) of pale yellow crystals of III, m.p. 101–104.5°.

A sample of II, from bromination of I, was dissolved in boiling aqueous sodium bicarbonate (using Norite) and the filtered solution chilled and filtered to remove yellow plates of sodium 3-bromotropolone.[2] This was dissolved in the minimum amount of hot water and the solution acidified to *p*H 2. Chilling and filtering gave pale yellow crystals of III, m.p. 105–105.5°; Cook, *et al.*,[2] give m.p. 103–106°; Nozoe, *et al.*,[12] give m.p. 107–108°.

Anal.[11] Calcd. for $C_7H_5BrO_2$: C, 41.8; H, 2.5; Br, 39.8. Found: C, 41.5; H, 2.7; Br, 39.9.

The ultraviolet absorption spectra in chloroform show principal maxima at 257.5, 328.5, 264 and 381.5 mμ, ε 37,800, 7,450, 7,000 and 7,250, respectively. The infrared spectra in Nujol mull in the 6–8 μ region show bands at 6.24, 6.28, 6.48, 6.78, 7.09, 7.35, 7.67 and 8.07 μ.

3-Bromotropolone hydrobromide (7.46 g.), from bromination of I, was refluxed in glacial acetic acid until evolution of hydrogen bromide ceased. The solution was evaporated to dryness *in vacuo* and the residue was recrystallized from cyclohexane to give 3.30 g. of pale yellow crystals of III, m.p. 102–106.5°. Recrystallization from aqueous acetic acid, using Norite, gave pale yellow crystals of III, m.p. 103.5–105.5°.

(12) T. Nozoe, Y. Kitahara, K. Yamane and A. Yoshikoshi, *Proc. Japan Acad.*, **27**, 18 (1951), find m.p. 107–108°, not 111° as reported in ref. 3.

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Paper Chromatography of the Anilides of Saccharinic Acids

BY JOHN W. GREEN

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Aldonic acids, as gluconic acid and the keto derivatives, have been separated on paper chromatograms by Norris and Campbell,[1] by Dyfverman, Lindberg and Wood,[2] and by Dyfverman.[3] The compounds were separated either as the free acids, or were first converted to the phenyl hydrazides. The various spots on the chromatogram were then detected by spraying the dried sheet with ammoniacal silver nitrate or with resorcinol in alcoholic hydrochloric acid. A new technique is now suggested, whereby the aldonic or saccharinic acids are first converted to the anilides, and then detected on the chromatogram by the quenching effect of the aromatic grouping on the ultraviolet fluorescence of the dye Rhodamine B. This dye has been suggested by Meigh[4] for the detection of aromatic nitro compounds on the paper chromatogram.

Paper chromatography of the crude saccharinic anilides obtained from D-glucose (or D-mannose) and D-galactose gives three very pronounced spots of R_f 0.60, 0.86 and 0.92, approximately, and a very faint spot of R_f 0.75. The crude saccharinic anilides of the two pentoses, D-xylose and L-arabinose, give three distinct spots of R_f 0.75, 0.86 and 0.92, approximately. Fractionation of mixtures on a cellulose column[5] gave two crystalline fractions—a C_6-saccharinic anilide of R_f 0.60 (obtained from D-glucose originally) and a C_5-saccharinic anilide of R_f 0.75 (obtained from L-arabinose originally). The identity of these compounds is being investigated.

This method shows promise of being a valuable tool for analyzing mixtures of saccharinic acids formed by the action of alkali on reducing sugars and polysaccharides (*i.e.*, alkaline pulping processes). It is being studied further and details will be published shortly.

Experimental

Preparation of Aldonic Anilides.—A solution of 0.1 g. of aldonic acid or lactone in 0.5 cc. of water is treated with 10 cc. of absolute ethanol, 0.2 cc. of aniline and 0.1 cc. of glacial acetic acid, and the solution concentrated to dryness on the steam-bath in 30–60 minutes. Solution of the residue, in the case of gluconic 1,5-lactone, in 15 cc. of 60% ethanol and visual comparison on a paper chromatogram with "known" spots of pure gluconic anilide showed almost complete conversion to the anilide. The rotation of the 60% ethanol solution checked within 5% of the value calculated for complete conversion. Use of ethanol and aniline alone, without the acetic acid, gave slightly lower yields. Gluconic 1,4-lactone gave slightly lower yields than did the 1,5-lactone.

Preparation of Saccharinic Anilides.—The given sugar is heated in 8 *N* NaOH for 8 hours on the steam-bath,[6] the alkaline solution diluted to 10 volumes and passed through a column of Amberlite IR-120 cation-exchange resin to remove the Na^+ ions, and the effluent concentrated *in vacuo* at 45°. The resulting sirup, containing crude saccharinic acids, is converted to the anilides as above, and then dissolved in 95% ethanol for spotting on the chromatographic paper.

Paper Chromatography of the Anilides.—A 7 by 24 inch sheet of Whatman No. 1 paper is spotted with 4 microliters of 1% aldonic anilide in 60% ethanol or 1 microliter of 5% crude saccharinic anilides in 95% ethanol. The mobile solvent used is a 9:1:2 v./v. mixture of acetone–water–benzene containing 4 mg. of Rhodamine B dye per 100 cc. The solvent is allowed to run the full length of the sheet (21 inches from the starting line), the paper is air-dried and then viewed under an ultraviolet light source.[7] The anilides show up as dark spots on a light yellow background. (The dye moves almost the full distance of solvent travel, so that substances with R_f values up to 0.97 can be detected. Tests with gluconic and other aldonic anilides show that 0.01 mg. can be easily detected.)

The aldonic anilides[8] were separated into C_6 and C_5 groupings, but there was little separation between stereoisomers. The R_f values also increased slightly with increasing length of solvent advance. Thus, for a solvent advance of 21 inches, the R_f values, measured at the center of each spot, were: D-galactonic anilide, 0.61; D-gluconic anilide, 0.63; D-mannonic anilide, 0.64; L-arabonic anilide, 0.70; D-xylonic anilide, 0.71; L-rhamnonic anilide, 0.82.[9]

(1) Flora C. Norris and J. R. Campbell, *Can. J. Research*, **27c**, 253 (1949).

(2) A. Dyfverman, B. Lindberg and D. Wood, *Acta Chem. Scand.*, **5**, 253 (1951).

(3) A. Dyfverman, *ibid.*, **7**, 280 (1953).

(4) D. F. Meigh, *Nature*, **169**, 706 (1952).

(5) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

(6) J. U. Nef, *Ann.*, **376**, 1 (1910).

(7) Mineralite Model SL 2537, obtained from the Ultra-Violet Products Co., 145 Pasadena Avenue, South Pasadena, California.

(8) E. Fischer and F. Passmore, *Ber.*, **22**, 2728 (1889); E. Kohn, *Monatsh. Chem.*, **16**, 333 (1895); Th. Van Marle, *Rec. trav. chim.*, **39**, 549 (1920).

(9) *d*-Gluconic and *d*-galactonic phenylhydrazides were also run on paper with the above solvent. The R_f values were similar to those for the anilides, so no better separation was achieved. The 60% ethanol solutions of the phenyl hydrazides tend to color on standing, but the anilide solutions showed no color after 1 month.

Fractionation of the Saccharinic Anilides on a Cellulose Column.—A concentrated ethanol solution containing the saccharinic anilides formed from 1 g. of sugar was applied to the top of a 2 by 22 inch cellulose column (Whatman Cellulose powder) and washed with about 2 liters of 9:1:2 acetone–water–benzene during a period of 20 hours. The first 500 cc. of effluent were discarded, and then 12-cc. fractions taken every 7.5 minutes. The anilides were eluted very rapidly, the slowest fraction, of R_f 0.60, being found in fractions 48 to 56. The **C_6-saccharinic anilide**, isolated from a mixture prepared from D-glucose, was obtained in 150 mg. crude and 60 mg. purified yield, m.p. 120–121° cor. (crystallized from ethyl acetate and ligroin), $[\alpha]^{23}D$ −23.9° (95% EtOH, *c* 3.3). Calcd. for $C_{12}H_{17}O_6N$: C, 56.50; H, 6.70; N, 5.48. Found: C, 56.41; H, 6.74; N, 5.38. The **C_5-saccharinic anilide**, isolated from a mixture prepared from L-arabinose, was obtained in 10 mg. yield, m.p. 108–110° cor. (crystallized from ethyl acetate–benzene, and then benzene–ligroin). It is quite soluble in ether. No rotation was taken. Calcd. for $C_{11}H_{15}O_4N$: C, 58.70; H, 6.70; N, 6.22. Found: C, 59.30; H, 7.33; N, 5.68.

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Glucoluteolin Isolated from the Leaves of *Sophora angustifolia*

BY SHIZUO HATTORI AND HIROAKI MATSUDA

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Sophora angustifolia Siebold et Zuccarini is a perennial herbaceous plant of the family *Leguminosae* which is very common in Japan. Its root contains the alkaloid matrin.[1]

From the concentrated aqueous extract of the leaves of this plant, ethyl acetate extracted a pale yellow glycoside, which was purified by recrystallization from pyridine–water and melted at 254°. On hydrolysis, it yielded the luteolin (5,7,3′,4′-tetrahydroxyflavone) and the sugar glucose. By direct comparison, this glycoside proved to be identical with glucoluteolin which we had isolated from the leaves of *Humulus japonicus* Siebold et Zuccarini[2] and has the structure luteolin-7-glucoside.

Experimental

Twelve kg. of fresh leaves, collected in June, was extracted with 10 l. of boiling water; the decoction was concentrated to 1 l. and after extraction with ether repeatedly extracted with ethyl acetate. On concentration of the combined yellow ethyl acetate solution by distillation a yellow residue was obtained.

Pale yellow needles, m.p. 233–234°, yield 0.02%, resulted on recrystallization several times from ethanol. The crystals were further purified by recrystallization from water on addition of pyridine and obtained as somewhat lustrous, pale yellow needles of m.p. 254°. An ethanolic solution of this substance gave a greenish-brown coloration, was easily oxidized by means of pentamminecobaltic chloride, showed strongly positive Molisch reaction, and, when reduced with magnesium powder and concd. hydrochloric acid, gave an orange-yellow color. The R_f is 0.47 in *n*-butyl alcohol–acetic acid–water (4:1:1). All of these properties were the same as those exhibited by authentic glucoluteolin ($C_{21}H_{20}O_{11}$) and no lowering occurred in a mixed melting test with the authentic sample.

The crystals are very difficultly soluble in hot water and dilute ethanol, and contain 6% (1.5 moles) of water of crystallization.

It is very difficult to hydrolyze glucoluteolin completely. The hydrolysis was achieved by heating it with 30% sulfuric acid for 6 hours. After cooling, the precipitated aglycone was filtered (yield, about 55% of the glycoside used) and when recrystallized from ethanol, yellow needles gradually separated. These crystals of the aglycone did not melt under 300°, showed a dark greenish-brown coloration and, when reduced with magnesium powder and concd. hydrochloric acid, gave an orange color.

Part of the aglycone was acetylated by the usual method and the purified acetate consisted of white long needles of m.p. 222°. This melting point did not alter when the acetylated product was mixed with authentic tetraacetylluteolin. Further, the methylated aglycone, which was obtained by methylating with dimethyl sulfate and potassium carbonate in acetone, consisted of colorless needles and melted at 144°. When mixed with authentic tetramethylluteolin, the methylated product did not lower the melting point.

By means of osazone formation, glucose was identified as the only sugar in the hydrolysate.

The methylated glucoside, which was obtained by methylating the glucoluteolin with dimethyl sulfate and potassium carbonate in acetone, gave, on hydrolysis with boiling 30% sulfuric acid, colorless needles of m.p. 284°. These were identical with authentic 5,3′,4′-trimethylluteolin by mixed melting test.

Not only the leaves, but also the pale yellow flowers of this plant contain glucoluteolin. In this latter case, however, 50% ethanol was found to be more convenient than hot water for its extraction. The yield was a little higher, that is, 0.06% of the fresh material.

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(1) N. Nagai and H. Kondo, *J. Pharmac. Soc. Japan*, **23**, 993 (1903).

(2) S. Hattori and H. Matsuda, *Acta Phytochimica (Japan)*, **15**, 233 (1949).

Derivatives of Reserpine. Communication on the Rauwolfia Alkaloids. XIII

BY CHARLES F. HUEBNER

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We wish to report some new derivatives of reserpine[1] prepared with the object of determining the effect of chemical change on pharmacological activity. Chemical modifications of reserpine (I) resulted in compounds obtained from alkylation of the indole nitrogen (II and III) and by conversion of the carbomethoxy group to the corresponding amide IV.

Methyl reserpate, in the form of its N-potassium derivative, readily undergoes alkylation in liquid ammonia with methyl iodide and with allyl bromide according to an adaptation of the method recently described for the preparation of N-methylharman.[2] The N-alkyl methyl reserpates are then esterified with 3,4,5-trimethoxybenzoyl chloride (II and III). Reserpine itself, can be directly N-alkylated; however, this is the less convenient of the two alternative methods because of the relative insolubility of reserpine and its N-potassium derivative in liquid ammonia. The amide of reserpic acid results from the action of a large excess of sodium amide on reserpine in liquid ammonia, ammonolysis occurring at both its ester linkages. Reesterification of reserpamide with 3,4,5-trimethoxybenzoyl chloride gives the amide corresponding to reserpine (IV).

Pharmacological investigation of these reserpine derivatives by Dr. Plummer and his associates showed that they are devoid of the tranquillizing and hypotensive properties characteristic of the

(1) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

(2) F. A. L. Anet, D. Chakravarti, R. Robinson and E. Schlittler, *J. Chem. Soc.*, 1242 (1954); see also H. Plieninger, *Ber.*, **87**, 127 (1954).