Summary and Conclusions

Three new reactions of fatty isocyanides were discovered and are summarized below.

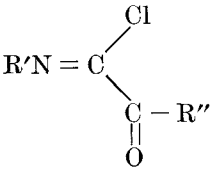
a) With halogens, products of the type $(RNCX)_n$ were isolated; this differs from the aromatic or short-chain isocyanides which form $RNCX_2$ under similar conditions.

b) With alkylating agents, such as methyl chloride or ethyl sulfate, the fatty isocyanides formed quaternary-like surface-active derivatives. This type of reaction has not been reported previously.

c) With acid chlorides, the following type of structures were formed:

$$\begin{pmatrix} R''C=O \\ | \\ R'N=C \end{pmatrix}^{+} Cl^{-}$$

this differs from the aromatic or short-chain isocyanides which are reported to form



under similar conditions.

In reactions of fatty isocyanides with alkylating agents and with acid chlorides evidence was presented to indicate that addition occurred at the nitrogen of the isocyanide group. These are the first examples in the literature of such reactions by isocyanides.

REFERENCES

1. Gautier, A., Ann., *151*, 239 (1866).
2. Nef, J. V., *ibid.*, *270*, 274 (1892).
3. Nef, J. V., *ibid.*, *287*, 265 (1895).
4. Passerini, M., Gazz. chim. ital., *56*, 365 (1926).
5. Passerini, M., *ibid.*, *53*, 410 (1923).
6. Gilman, H., "Organic Chemistry," 2nd ed., vol. 2, pp. 1907–1908, John Wiley and Sons, New York, 1947.
7. Whitmore, F. C., "Organic Chemistry," 1st ed., pp. 505–506, D. Van Nostrand Company Inc., 1938.
8. Baker, R. H., and Stanonis, D., J. Am. Chem. Soc., *73*, 699 (1951).
9. McBride, J. J., and Berchell, H. C., *ibid.*, *74*, 5247 (1952).

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Identification of Rutin and Isoquercitrin in Cottonseed[1]

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BOATNER (1) has stated that, although many of the pigments of the cotton flower have been isolated and identified as flavonoid compounds, there is little evidence that these or related pigments occur in the seed. Gurevich (2) reported microchemical tests which suggested the presence of flavones or closely related pigments in the epidermal cells of the cotyledons above the palisade cells. Goldvoskii (3), also Boatner (1), pointed out however that before one could draw any definite conclusions concerning the presence of flavonoid compounds in cottonseed, one would need to supplement the microchemical tests with the isolation and identification of these pigments. This present paper reports the separation and identification of two such pigments.

Paper chromatography of isopropyl alcohol extracts of crushed, delinted cottonseed (kernel and hull) in our laboratory has revealed the presence of at least six pigments which preliminary tests indicate to be flavonoid compounds. We have therefore undertaken studies to identify the individual flavonoid pigments present in the cottonseed. Two of these have been separated and identified as isoquercitrin (quercetin-3-glucoside) and rutin (quercetin-3-rhamnoglucoside). Quercetin is 3,3′,4′,5,7-pentahydroxyflavone.

Experimental

Extraction and Preliminary Separation of Pigments. Delinted cottonseed (1 kg.), obtained through the courtesy of the National Cottonseed Products Association Inc. from a cottonseed oil producer in Lubbock, Tex., was crushed with a food blendor and extracted in Soxhlet extractors with a total of 3 l. of 85% isopropyl alcohol-water until the solvent in contact with the seed no longer gave a bright yellow color. This extract was concentrated to 200 ml. *in vacuo*, then transferred to a beaker. To it were added 100 ml. of hot distilled water, with stirring, so that the oil collected together. The mixture was kept in the refrigerator (5–10°C.) for 3 hr. The lower layer was decanted and concentrated to 25 ml.; 50 ml. of 95% ethyl alcohol were added to the concentrate. The resulting solution was streaked onto 24 sheets of Whatman No. 3 MM chromatography paper, 18½ in. × 22¼ in., and the chromatograms were developed for 18–20 hr. in n-butyl alcohol-acetic acid-water (6:1:2 v./v.). Three major zones that fluoresced brown in long wavelength ultraviolet light (3660 Å) were observed. Rutin was one of the compounds present in the second brown zone (called zone 2) from the top, with an approximate R_f value of 0.39. Isoquercitrin was present in zone 3, with R_f value of approximately 0.59.

Separation of Rutin. Zone 2 from the n-butyl alcohol-acetic acid-water chromatography was cut from each paper, minced, and extracted in a Soxhlet extractor with 95% ethyl alcohol. The extract was concentrated *in vacuo* to 25 ml. and streaked onto sheets of Whatman 3 MM paper. The chromatograms were developed in 15% acetic acid-water for 5–6 hr. Three brown fluorescent zones resulted from the one original zone 2. The brown fluorescent zone with the lowest R_f value (about 0.61) of these three contained the rutin. Identification studies on the compounds of the other two zones are in progress.

[1] This manuscript has been taken in part from a thesis submitted by Charles Pratt for the M.S. degree in Chemistry, University of Oklahoma, August 1958.

[2] Aided by a Fellowship from the National Medical Fellowships Inc. through funds appropriated by the National Foundation for Infantile Paralysis.

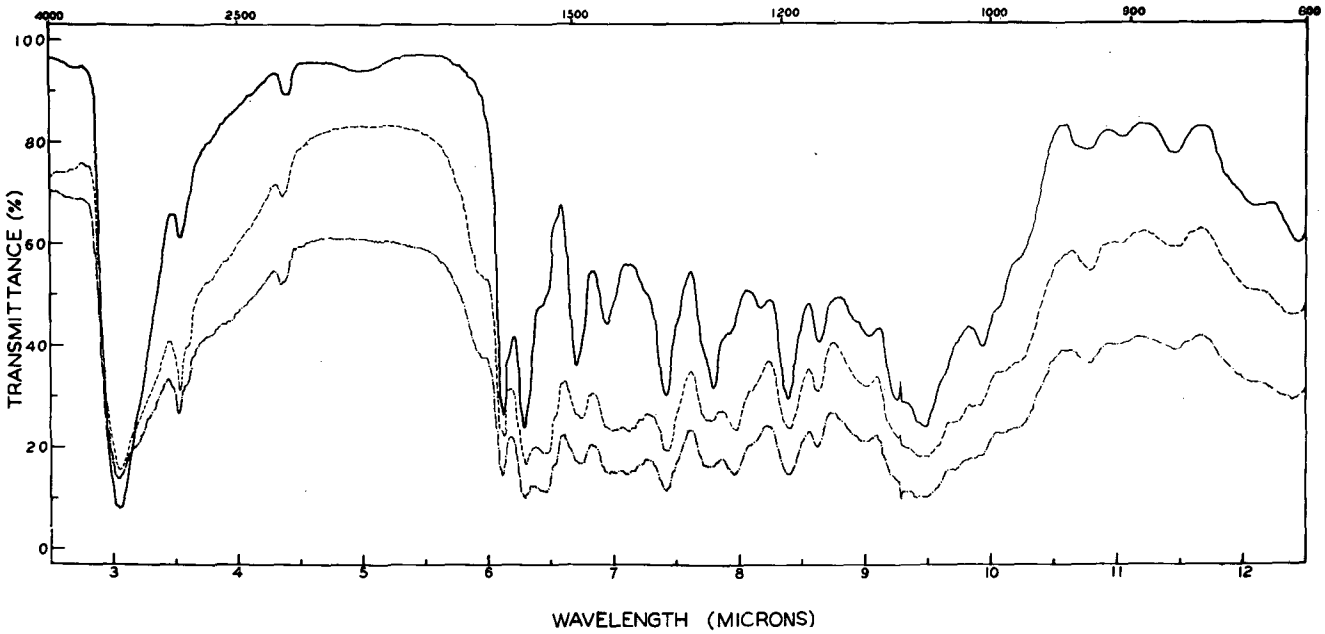

Fig. 1. Infrared spectra of rutin samples.
———— authentic rutin, crystals
- - - - - - - - - - authentic rutin from paper
—.—.—. cottonseed compound "2A-2" from paper.

After air-drying, the zone containing rutin was cut from all the papers, minced, and extracted with methyl alcohol-water (3:1). The resulting extract was concentrated and streaked on Whatman No. 3 MM filter paper. The chromatograms were chromatographed a second time in the 15% acetic acid-solvent system. Two brown fluorescing zones were present. The zone with the higher R_f of these two was cut from every paper and eluted with the methyl alcohol-water solution. The eluate was concentrated to a small volume, then investigated further for proof of identity. On all further paper chromatography comparisons this compound (called "2A-2") corresponded with authentic rutin.

Separation of Isoquercitrin. Each zone 3 from the n-butyl alcohol-acetic acid-water chromatograms was cut out, extracted with methyl alcohol-water in a Soxhlet, then concentrated. The concentrate was streaked on Whatman No. 3 MM paper, and the chromatograms were developed with the 15% acetic acid-water solvent. Three major zones appeared. The brown fluorescing zone with R_f value of approximately 0.46 contained the isoquercitrin. The other compounds have not yet been identified. The zone containing the isoquercitrin was cut out and extracted. The extract was concentrated, then streaked on chromatography paper. The chromatograms were developed a second time in the 15% acetic acid-water system for additional purification. The main brown fluorescing zone (called "3A") was eluted, concentrated, and subjected to studies involving proof of its identity as isoquercitrin.

Identification Studies. The chromatographically pure fractions "2A-2" and "3A" were each checked against authentic samples of rutin and isoquercitrin on one- and two-dimensional paper chromatograms, using the solvent systems n-butyl alcohol-acetic acid-water (6:1:2 v./v.), 15% acetic acid-water, 60% acetic acid-water, and nitromethane-benzene-water (2:3:5 v./v.) alone and in combinations. In every case, compound "2A-2" corresponded to rutin and compound "3A" to isoquercitrin. Two-dimensional mixed chromatograms of "2A-2" superimposed on the authentic rutin, using the n-butyl alcohol-acetic acid-water system in one direction and the 15% acetic acid-water system in the second direction, gave only one spot. Likewise compound "3A" was found to be identical with authentic isoquercitrin on such mixed two-dimensional paper chromatograms.

Color reactions of compounds "2A-2" and "3A" checked with those obtained with authentic rutin and isoquercitrin, respectively (4).

Determination of the ultraviolet absorption spectra of compounds "2A-2" and "3A" in ethyl alcohol, using the Beckman spectrophotometer, Model DU, gave the same curves as those obtained for authentic rutin and isoquercitrin, respectively, and the same as previously reported in the literature (5, 6).

The infrared absorption spectrum of compound "2A-2" (KBr pellet) as determined on the Perkin-Elmer recording infrared spectrophotometer, Model 21, was essentially the same as those for authentic rutin samples (Figure 1). Likewise the infrared absorption spectrum of compound "3A" was the same as that of authentic isoquercitrin.

Hydrolysis Products of the Two Glycosides. Each glycoside (10 mg.) was dissolved in 10 ml. of 95% ethyl alcohol and separately hydrolyzed with 20 ml. of 2% H_2SO_4 (by wt.) by refluxing for 2 hr. The hydrolysates, after concentration *in vacuo*, were placed in the refrigerator over-night, then filtered. The precipitate in each case was dissolved in 95% ethyl alcohol and compared with authentic quercetin which had been subjected to paper chromatography in the solvent systems mentioned previously. In every case and with every chromogenic spray reagent tried, the aglycones from the glycosides "2A-2" and "3A" checked with each other and with authentic quercetin (4).

Each filtrate which remained after removal of the

aglycone was passed through a column containing Dowex 3 (20–50 mesh) underneath and Dowex 50 (100 mesh) on top. The Dowex 3 had been washed with 20% NaOH solution, followed by thorough washing with distilled water to neutrality. The Dowex 50 had been washed with 5% H_2SO_4 solution, then with distilled water. Each filtrate was passed through the resin separately. Each effluent solution was concentrated under reduced pressure to 1 ml. Identification was accomplished on paper chromatograms by comparing the unknowns with known samples of sugars, using as the solvent system n-butyl alcohol-pyridine-benzene-water (5:3:1:3 v./v.) and using the phosphate salt of 2-aminodiphenyl in glacial acetic acid as a chromogenic spray (7).

In the hydrolysate from compound "2A-2" (rutin), glucose and rhamnose were present. In the hydrolysate from compound "3A" (isoquercitrin), glucose was identified as the only sugar present.

For determination of the ratio of each sugar to the aglycone present per molecule of glycoside, the hydrolysate was concentrated *in vacuo,* and the remaining suspension was set in the refrigerator over-night. It was then filtered on a sintered glass funnel. The filtrate was passed through the column containing the Dowex resins, as previously described, and concentrated to about 3 ml. under reduced pressure, then the concentrate was diluted to 5 ml. in a volumetric flask. An aliquot (0.4 ml.) was streaked on Whatman No. 1 paper and analyzed quantitatively by a method based on the procedure reported by Timell *et al.* (7).

The precipitate from the hydrolysate was dissolved in 95% ethyl alcohol, and the solution was contrated *in vacuo.* The solution volume was then adjusted to 5 ml. An aliquot (0.5 ml.) was chromatographed, using the n-butyl alcohlo-acetic acid-water system. The quercetin zone was cut out and eluted with ethyl alcohol-water (4:1) for 24 hr. The eluate was diluted to 10 ml. A blank strip was run in the same manner except that no quercetin was present. The absorbance of each quercetin solution was measured at 375 mμ, using a 1-cm. silica cell and a Beckman spectrophotometer, Model DU. The quantity of quercetin present was determined from a standard quercetin curve. To obtain the standard quercetin curve, five standard samples of different, but known, concentrations of quercetin were processed through exactly the same procedure as already described for quercetin in the glycoside hydrolysate. A straight-line standard curve was obtained by plotting the absorbance against micrograms of quercetin originally streaked at the beginning of its paper chromatography.

By these procedures, compound "2A-2" (rutin) was found to have a ratio, within experimental error, of one quercetin to one glucose to one rhamnose whereas compound "3A" (isoquercitrin) had a ratio, within experimental error, of one quercetin to one glucose.

Summary

The flavonol glycosides, rutin and isoquercitrin, have been separated from crushed, delinted cottonseed (kernel and hull) by extensive use of paper chromatography. The identification of these flavonoid pigments has been achieved through paper chromatography, ultraviolet and infrared spectrophotometry, and qualitative and quantitative analysis of their hydrolysis products. Details of the separation and identification have been described.

Acknowledgments

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REFERENCES

1. Boatner, Charlotte H., "Pigments of Cottonseed," Chapter VI, "Cottonseed and Cottonseed Products," ed. by Bailey, Alton E., Interscience Publishers Inc., New York, 1948.
2. Gurevich, M., Vsesoyuz Nauch. Issledovatel. Inst. Zhirov. 31–42 (1936), as quoted by Boatner in (1).
3. Goldovskii, A. M., Vsesoyuz. Nauch. Issledovatel. Inst. Zhirov, 5–31 (1936), as quoted by Boatner in (1).
4. Gage, T. B., Douglass, C. D., and Wender, S. H., Anal. Chem., *23,* 1582–1585 (1951).
5. Douglass, C. D., Howard, W. L., and Wender, S. H., J. Am. Chem. Soc., *71,* 2658–2659 (1949).
6. Griffith, J. Q. Jr., Krewson, C. F., and Naghski, J. "Rutin and Related Flavonoids," Chapter II, Mack Publishing Company, Easton, Pa., 1955.
7. Timell, T. E., Glaudemans, C. P. J., and Currie, A. L., Anal. Chem., *28,* 1916–1920 (1956).

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Reactions of Dienophiles with Vegetable Oils. I. Reactions of Maleic Esters with Sulfur Dioxide Catalyst[1]

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PREVIOUS WORK in these laboratories (2, 8) has been concerned with the Diels-Alder addition of dienophiles to conjugated fatty acids. In that work linoleic acid was isomerized to produce *cis,trans*-conjugated acid. After elaidinization to the *trans,trans* form these acids were treated with various dienophiles to give adducts of the type I. These adducts and their derivatives have potential utility as plasticizers (2). This potential may be limited however by the cost of obtaining the *trans,trans*-conjugated acids from vegetable oils. Further work (6) showed that *cis,trans*-acids could be used in these reactions if selenium were present as a catalyst. The next logical step is the preparation of adducts having structure I directly from vegetable oils. Adducts of the whole oil might have properties superior to those of the simple ad-

[1] Presented at fall meeting, American Oil Chemists' Society, Chicago, Ill., October 20–22, 1958.
[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.