grams of 3B-2A-2, superimposed on the authentic kaempferol-3-rhamnoglucoside, 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.

Determination of the ultraviolet absorption spectrum of 3B-2A-2 in ethyl alcohol, using the Beckman DK-1 recording spectrophotometer, produced the same curve as that obtained for authentic kaempferol-3-rhamnoglucoside.

Identification of Quercetin-3-glucoglucoside. Ten mg. of the chromatographically-pure 3B-2A-1 was hydrolyzed by the procedure used for the kaempferol glycoside. By paper chromatography the products were found to be quercetin and glucose.

R_f values of 3B-2A-1 before hydrolysis (Table 1) indicated that this glycoside of quercetin likely contained 2, rather than 1 or 3, units of monosaccharide. For proof of the ratio of glucose to quercetin in this glycoside another 10 mg. of 3B-2A-1 were hydrolyzed as described previously except that the organic layer was evaporated to dryness and then dissolved to make 5 ml. of solution in 95% ethyl alcohol. The neutral aqueous layer was concentrated to exactly 2 ml. An aliquot (0.6 ml.) of the aqueous solution was streaked on Whatman No. 1 chromatography paper and analyzed quantitatively by a modified procedure of Timell (6).

Of the 5-ml. solution of aglycone in 95% ethyl alcohol, an aliquot (0.4 ml.) was chromatographed, using the n-butyl alcohol-acetic acid-water system. The quercetin zone was cut out and eluted with 95% ethyl alcohol and made to exactly 10 ml. in volume. A blank chromatography paper strip was treated by the same procedure except that no quercetin was present. The absorbance of the quercetin was measured against its blank at 374 mμ, using 1-cm. silica cells and the Beckman spectrophotometer, Model DU. The quantity of quercetin present was determined from a standard quercetin curve. To obtain this standard curve five samples of different, but known, concentrations of quercetin were processed through the same procedure already described for the quercetin in the glycoside hydrolysate. A straight-line curve was obtained by plotting the absorbance against micrograms of quercetin originally streaked at the beginning of its paper chromatography. By this method, glycoside 3B-2A-1 was found to have a ratio, within experimental error, of one quercetin to two glucose units.

Spectral Studies. In order to locate the position or positions of attachment of the two glucose units a spectral shift study was made by the procedures of Jurd (7) and of Jurd and Horowitz (8). Since the aglycone was quercetin, the sugar linkages could occur at positions 3,3′,4′,5, or 7 of quercetin. First, the ultraviolet spectrum of compound 3B-2A-1 in absolute ethyl alcohol was determined by using the Beckman DK-1 recording spectrophotometer. Maxima were at 260 mμ and 370 mμ. For analysis of position 7 excess anhydrous fused sodium acetate was added to the sample cell and to the blank, and, after 5–10 min. the spectrum was determined again. The first maximum had shifted from 260 mμ to 272 mμ. This indicated that the Number 7 hydroxyl group was not substituted by a glucose unit.

To 2 ml. of the 3B-2A-1 stock solution (approximately 0.0001 M) in absolute ethyl alcohol were added 2 ml. of a saturated solution of boric acid in absolute ethyl alcohol. The solution was diluted to 10 ml. with absolute ethyl alcohol, and an excess of sodium acetate was added. After the solution was shaken and allowed to settle for 10–20 min., the spectrum of the solution was recorded on the same graph as the untreated sample. The 260 mμ peak had shifted 23 mμ toward shorter wavelengths. The 370 mμ maximum had shifted to 385 mμ. These shifts indicated that the 3′,4′-o-dihydroxy groups of the quercetin are not substituted by sugar in the glycoside studied.

The reaction of the glycoside with aluminum chloride indicated that the sugar was not on the Number 5 position. This leaves the Number 3 position as the point of attachment of glucose. Actually the substitution of the Number 3 hydroxyl group by the glucose had already been evidenced by the experimental fact that this quercetin glycoside fluoresces brown, rather than yellow, under long wavelength (3660 Å) ultraviolet light.

Acknowledgment

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REFERENCES

1. Hukuti, G., J. Pharm. Soc. (Japan), *59*, 85 (1939).
2. Hayashi, K., and Ouchi, K., Acta Phytochim. (Japan), *15*, 1 (1949).
3. Perkin, A.G., and Everest, A.E., "The Natural Organic Colouring Matters," Longmans, Green, and Company, London, England, 1918, p. 223.
4. Boatner, Charlotte H., Pigments of Cottonseed, Chapter VI, "Cottonseed and Cottonseed Products," ed. by A.E. Bailey, Interscience Publishers Inc., New York, 1948.
5. Pratt, Charles, and Wender, S.H., J. Am. Oil Chemists' Soc., *36*, 392 (1959).
6. Timell, T.E., Glaudemans, C.P.J., and Curie, A.L., Anal. Chem. *28*, 1916 (1956).
7. Jurd, L., Arch. Biochem. and Biophys., *63*, 376 (1956).
8. Jurd, L., and Horowitz, R.M., J. Org. Chem., *22*, 1618 (1957).

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Report of the Uniform Methods Committee, May, 1961

THE MEETING of the Uniform Methods Committee of the American Oil Chemists' Society was held in St. Louis on May 2, 1961. E.F. Sipos, R.A. Marmor, R.J. Houle, K.E. Holt, and J.H. Benedict (representing E.M. Sallee, editor *ex officio*), and D.L. Henry were present. Vistors were Edward Handschumaker, and T.D. Parks. The following matters were discussed and decisions were made as indicated:

Report of the Soap and Synthetic Detergent Analysis Committee, J.C. Harris, chairman—The Soap and Synthetic Detergent Analysis Committee recommended advancement of Tentative Method Da 17–52 and Dd 7b–55 to official status, and to correct Db 8–48 by making a change in section C. 1, line 2 Da 12–42 to Da 12–48. Their report was accepted by the Uniform Methods Committee, and the recommendations were approved.

Report of the Seed and Meal Analysis Committee, M.H. Fowler, chairman—The Seed and Meal Analysis Committee recommended the adoption of a revised Crude Fiber Method to replace Ba 6–49. Their report was accepted by the Uniform Methods Committee, and the recommendation was approved.

Report from the chairman of the Oxirane subcommittee,

K.E. Holt, chairman—This report requested that Method Cd 9–57 be revised to make Section B.4 show that potassium acid phthalate is used as a standard rather than sodium carbonate. This new standard is in general use for this method, and the Uniform Methods Committee recommends its adoption.

Report of the Nomenclature Committee, N.B. Knoepfler, chairman—The Nomenclature Committee strongly recommends that prompt steps be taken to encourage the use of chemically-descriptive nomenclature for fatty acids and to avoid the tendency to apply "trade" names in presentations and papers at meetings and in the Journal of this Society. The committee further recommends that, in the interest of educating and familiarizing the membership with proper nomenclature, seminars on the subject be included in programs for future meetings. This report was accepted by the Uniform Methods Committee, and their recommendations approved.

Report of the Statistical Committe, W.E. Link, chairman—The report of the Statistical Committee was accepted by the Uniform Methods Committee, which requests the Statistical Committee to investigate the needs for precision data of the current oil-refining methods.

Report of the Refining Committee, A.E. Blankenship, chairman—The report of the Refining Committee was accepted, and no changes in methods were recommended.

The report from the Executive Secretary's office, listing items distributed by the LaPine Scientific Company, shows these supplies to be in order; they are in sufficient quantities for current needs.

A request from the chairman of the Soybean Analyses Committee of the American Association of Cereal Chemists was received, asking that a joint committee (A.O.C.S.-A.A.C.C.) be established for the development of a standardized Protein Solubility Index Method. The Uniform Methods Committee recommends that an eight-member Liaison Committee be established, consisting of two representatives from each of the societies A.O.C.S., A.O.A.C., I.F.T., and A.A.C.C., with Endre Sipos as chairman. This committee is to study methods currently in use and to establish a uniform method.

Seeing a need that all thermometers be cross-indexed in the Methods H10–55 with A.S.T.M. thermometers, the Uniform Methods Committee requests the Methods editor to make all necessary additions.

J.J. GANUCHEAU
K.E. HOLT
R.J. HOULE
R.A. MARMOR
L.D. METCALF
ENDRE SIPOS
E.M. SALLEE, editor, *ex officio*
R.L. TERRILL
D.L. HENRY, chairman

Electrophoretic Fractionation of Soluble Antigenic Proteins from the Seed of *Ricinus Communis* (Castor Bean)

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A water-soluble, heat-stable protein component of castor seed meal was subjected to paper-strip electrophoresis in buffers of different chemical composition, pH values, and ionic strengths. It was shown that phosphate buffer at pH 7.4 to 8.0 and an ionic strength of approximately 0.05 gave a sharp resolution of castor seed proteins into bands which would bind bromophenol blue. Spies' Allergen CB-1A was shown to be resolved into six or more components at pH 8.0. Each major component band was found to be antigenic by passive cutaneous anaphylaxis in guinea pigs that were sensitized with rabbit antiserum to the crude castor seed protein preparation. Five bands were shown to be allergenic to humans.

The results appear to support earlier observations (1,2,10) that castor bean seed allergenicity to humans may be caused by more than one antigen in Spies' Allergen CB-1A and possibly by other antigens present in the seed but either absent from, or greatly reduced in concentration in, allergen CB-1A.

The water-soluble component of Altschul's active castor seed lipase was resolved into eight component bands, two of which did not appear in the electrophotograms of the heat-processed preparation CB-1A S.R.I.

IN PREVIOUS PAPERS (1,2) are described experiments which indicate that castor bean allergy may be caused by one or more of several antigens contained principally in the seed. The observations were made while the Schultz-Dale technique was used as directed by Coulson (3) for testing the fractions obtained by chromatography of castor seed allergen CB-1A on diethylaminoethyl-cellulose. It was found that uterine strips from guinea pigs that had been sensitized to allergen CB-1A would be discharged or rendered refractory to material from certain chromatogram peaks but would react maximally when challenged with material from subsequent peaks in the same chromatogram.

Such behavior appeared to indicate that the sensitized tissue contained more than one antibody to castor seed antigens and that significant separation of the antigens in fraction CB-1A had been accomplished by chromatography. Subsequent work indicated that certain of the chromatographic fractions were not electrophoretically homogeneous and that certain serial fractions appeared to have been contaminated by traces of material trailing from the preceding peak. Nevertheless the evidence did demonstrate the presence of more than one sensitizing antigen in castor allergen CB-1A and possibly several others in the seed flour and blossoms. These observations and the conclusions of Spies, Coulson, Stevens, *et al.* (4,5,6,7) that castor seed allergenicity is dependent upon a single specific basic protein, combined in different proportions with polysaccharide, suggested the desirability of fractionating the castor seed protein by another technique.

The technique of paper-strip electrophoresis is a simple procedure, in which there need be little or no loss of material since the constituent proteins are spread out into a "spectrum" or "profile" on a paper strip. Each component band of the electrophoretic profile may be cut out and tested for specific antigenic properties. By varying the conditions of ionic

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