

Figure 1. Diagrams of disc gel electrophoretic patterns of proteins from immature, mature, and germinating peanuts: (A) gel a, representative pattern of mature peanuts of Oklahoma-grown cultivars (*A. hypogaea* L. subsp. *fastigiata* var. *vulgaris*; Spanish botanical type; Comet, Starr, Argentine, or Spanhoma); (B) gels b–e, patterns of immature peanuts of Georgia-grown Florigiant (subsp. *hypogaea* var. *hypogaea*; Virginia botanical, Virginia market type) categorized into age groups 3 (b), 6 (c), 9 (d) and 12 (e) weeks; (C) gels f–i, four patterns that are representative of peanuts from Florigiant and also other cultivars (Spanish botanical type: Tifspan, Starr, Argentine, Spanscross, and Comet; Virginia botanical, Virginia market type: Virginia 56R, Virginia 61R, NC17, NC5, and NC2; and Runner market type: Early Runner, Florunner, Virginia Bunch 67) grown in one to four locations (Virginia, Georgia, Louisiana, and Texas); (D) gel j, pattern of germinating (24 hr) peanuts of Georgia-grown Florigiant.

improved cultivars with greater stability characteristics and more desirable patterns of nutrient components, it would be desirable to observe these factors closely. Such studies were initiated on the fatty acid composition of peanuts (Young *et al.*, 1972; Worthington *et al.*, 1972; Worthington and Hammons, 1971). Currently, similar studies involving proteins are under investigation in our laboratory.

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LITERATURE CITED

- Aldana, A. B., Fites, R. C., Pattee, H. E., *Plant Cell Physiol.* **13**, 515 (1972).
 Cherry, J. P., Dechary, J. M., Ory, R. L., *J. Agr. Food Chem.* **21**, 652 (1973).
 Cherry, J. P., Neucere, N. J., Ory, R. L., *J. Amer. Peanut Res. Ed. Ass.* **3**, 63 (1971).
 Cherry, J. P., Ory, R. L., *Phytochemistry* **12**, 283 (1973a).
 Cherry, J. P., Ory, R. L., *Phytochemistry* **12**, 1581 (1973b).
 Cherry, J. P., Ory, R. L., Mayne, R. Y., *J. Amer. Peanut Res. Ed. Ass.* **4**, 32 (1972).
 Hall, T. C., McLeester, R. C., Bliss, F. A., *Phytochemistry* **11**, 647 (1972).
 Holley, K. T., Hammons, R. O., *Univ. Ga. Coll. Agr. Exp. Sta. Res. Bull.* **32**, 1 (1968).
 Joo, P. S., Stotzky, G., *Can. J. Bot.* **48**, 1347 (1970).
 Kamra, O. P., *Z. Pflanzenzuecht.* **65**, 293 (1971).
 Nelson, O. E., *Advan. Agron.* **21**, 171 (1969).
 Ory, R. L., Cherry, J. P., *J. Amer. Peanut Res. Ed. Ass.* **4**, 21 (1972).
 Schenk, R. U., *Ga. Agr. Exp. Sta. Tech. Bull.*, N.S. **22**, 1 (1961).
 Worthington, R. E., Hammons, R. O., *Oleagineux* **26**, 695 (1971).
 Worthington, R. E., Hammons, R. O., Allison, J. R., *J. Agr. Food Chem.* **20**, 727 (1972).
 Young, C. T., Matlock, R. S., Waller, G. R., *J. Amer. Oil Chem. Soc.* **49**, 314 (1972).

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Production of 4-Hydroxy-5-methyl-3(2H)-furanone, a Component of Beef Flavor, from a 1-Amino-1-deoxy-D-fructuronic Acid

4-Hydroxy-5-methyl-3(2H)-furanone (a constituent of beef broth) is shown to be produced from 1-deoxy-1-dibenzylamino-D-fructuronic acid, which in turn was synthesized from the amine and D-glucuronic acid. The furanone can be obtained directly in the crystalline state by chloro-

form extraction of a reaction solution and was identified from its elemental analysis, infrared, ultraviolet, nuclear magnetic resonance, and mass spectra, as well as by comparison with authentic samples.

4-Hydroxy-5-methyl-3(2H)-furanone has been prepared in low yields by heating D-xylose (Severin and Seilmeier, 1968), D-ribose (Peer *et al.*, 1968a), and D-ribose 5-phosphate (Peer *et al.*, 1968b) with amine salts. The compound has a caramel-like or burnt aroma and its isolation from beef broth (Tonsbeek *et al.*, 1968) indicates that it is a component of the odor and flavor associated with cooked beef. From the known facts concerning such reactions (Hodge *et al.*, 1972), as well as the reaction conditions used in its preparation, it can be concluded that this furanone, as well as many other flavor and aroma constituents, is probably produced *via* a reaction involving con-

densation of an aldose with an amine to produce a 1-amino-1-deoxy-2-ketose (Amadori product) which subsequently dehydrates, with amine elimination, to give the desired product. It is noteworthy in this respect that the decomposition of Amadori products derived from many sugars which are normally found in foods has not been studied.

We have isolated and identified the title compound, which is produced, presumably as a decarboxylation product, from 1-deoxy-1-dibenzylamino-D-fructuronic acid which was prepared from dibenzylamine and sodium D-glucuronate (Heyns and Baltes, 1960).

EXPERIMENTAL SECTION

Proton magnetic resonance spectra were obtained on a Varian T-60 spectrometer and ^{13}C magnetic resonance spectra were obtained on a Bruker HX90 spectrometer. Ultraviolet spectra were obtained on a Coleman Model 124 recording double beam spectrophotometer and mass spectra were obtained using a CEC Model 21-110C mass spectrometer. Thin layer chromatography was performed using silica gel HF as the support and chloroform-methanol-acetic acid (90:10:2, v/v) as the irrigant. Spots were identified by ultraviolet irradiation or by spraying with 10% ethanolic sulfuric acid followed by heating at 110° for 10 min.

For the preparation of the furanone, 10 g of 1-deoxy-1-dibenzylamino-D-fructuronic acid (Heyns and Baltes, 1960) was suspended in a solution composed of 160 ml of water and 20 ml of acetic acid. The suspension was heated for 60 min at 100° , during which time the carbohydrate dissolved completely. After cooling, the solution was percolated through 200 ml of Dowex 50 (hydrogen form) and extracted four times with 100-ml portions of chloroform. After drying over anhydrous sodium sulfate, the chloroform was evaporated to dryness and the resulting crystalline mass was isolated on a filter, washed with hexane, and purified by sublimation at 60° and 0.25 mm; yield, 0.7 g.

RESULTS AND DISCUSSION

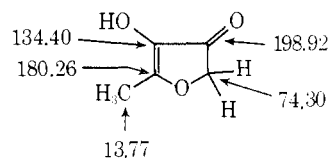
The dehydration products produced in aqueous acidic solution from either hexoses or Amadori products derived from them are the same; that is, the major product is 5-(hydroxymethyl)-2-furaldehyde (Gottschalk, 1952; Feather and Russell, 1969). Hexuronic acids when heated with aqueous acid give rise to 2-furaldehyde and 1,2-dihydroxycyclopenten-3-one (reductic acid as decarboxylation products and 5-formyl-2-furoic acid as a direct dehydration product) (Feather and Harris, 1966). On treating 1-deoxy-1-dibenzylamino-D-fructuronic acid with aqueous acid, a number of compounds were produced as evidenced by thin layer chromatography, but only traces of the normally observed dehydration products were observed.

The following data were collected for the crystalline compound: mp 129° ; ultraviolet λ_{max} 284 nm (ϵ 9600) (water); pmr signals (in deuterium oxide relative to TMS) at δ 2.28 (three protons), 4.65 (two protons), and 6.90 (br, one proton); major mass spectral peaks at m/e 43 (100%), 55 (16%), and 114 (37%). *Anal.* Calcd for $\text{C}_5\text{H}_6\text{O}_3$: C, 52.63; H, 5.32; N, 0.00. Found: C, 52.27; H, 5.48; N, 0.14. These values are, within experimental error, identical with those reported by Tonsbeek *et al.* (1968) for 4-hydroxy-5-methyl-3(2H)-furanone.

When the compound was compared with authentic samples of 4-hydroxy-5-methyl-3(2H)-furanone, all samples had identical thin layer chromatographic properties, low resolution mass spectra, and infrared spectra.

The ^{13}C nmr spectrum of the material is also consistent with its structure. The ^{13}C broad-band proton-decoupled Fourier transform nmr spectrum of a 10% solution of the furanone in water exhibited five peaks at 13.77, 74.30, 134.40, 180.26, and 198.92 ppm downfield from tetramethylsilane. The off-resonance proton decoupled ^{13}C spectrum (which shows carbon-hydrogen coupling) demonstrated that the 13-ppm peak was coupled to three protons, that a methyl group, the 74-ppm peak, was coupled to two protons (hence a methylene group), and that the carbons giving rise to the lower three peaks had no hydrogens bound to them. The chemical shift of the methylene

carbon indicated it was bound to oxygen and one other electron-withdrawing function. The peak at 198.9 was clearly due to a carbonyl. The field strength of this signal was too low to be an ester or an acid but characteristic of an α,β -unsaturated ketone (Stothers, 1972, p 238). The signal at 134.4 is clearly in the olefinic region (Stothers, 1972, p 190) indicating that the remaining resonance at 180.6 must also be part of such a system. On the basis of typical chemical-shift additivity relationships and the chemical shifts of the substituted ethylenes (Stothers, 1972, p 162) the 180-ppm carbon is bound to the furan oxygen and the CH_3 and the 134-ppm carbon is bound to HO and C(=O). In summary, then, the assignments are



This is the first production of the furanone from a sugar other than a pentose. The fact that it could be produced from a hexuronic acid greatly increases the number of potential sources for its production during the cooking of meat. Hexuronic acid containing compounds such as heparin and blood group polysaccharides as well as certain glycoproteins such as chondroitin sulfate (which contains up to 20% uronic acid) and hyaluronic acid (Marshall and Neuberger, 1970) all become probable sources for its production.

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LITERATURE CITED

- Feather, M. S., Harris, J. F., *J. Org. Chem.* **31**, 4018 (1966).
 Feather, M. S., Russell, K., *J. Org. Chem.* **34**, 2650 (1969).
 Gottschalk, A., *Biochem. J.* **52**, 455 (1952).
 Heyns, K., Baltes, W., *Chem. Ber.* **93**, 1616 (1960).
 Hodge, J. E., Mills, F. D., Fisher, B. C., *Cereal Sci. Today* **17**, 34 (1972).
 Marshall, R. D., Neuberger, A., *Advan. Carbohydr. Chem.* **25**, 407 (1970).
 Peer, H. G., Van Den Ouweland, G. A. M., De Groot, C. N., *Recl. Trav. Chim. Pays-Bas* **87**, 1011 (1968a).
 Peer, H. G., Van Den Ouweland, G. A. M., De Groot, C. N., *Recl. Trav. Chim. Pays-Bas* **87**, 1017 (1968b).
 Severin, Th., Seilmeier, W., *Z. Lebensm. Unters. Forsch.* **137**, 4 (1968).
 Stothers, J. B., "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972.
 Tonsbeek, C. H. T., Plancken, A. J., Weerdhot, T. V. D., *J. Agr. Food Chem.* **16**, 1016 (1968).

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