Steric hindrance of molecules, coulomb's static force and other factors are thought to be related to the formation of organic complex, and the above-mentioned results show that the value of parameter λ, showing energy level of molecular orbitals, will become in future an indicator to find out stabilizers or connective auxiliary solubilizing agents of pharmaceutical preparations forming complex in solutions. In fact, it is thought that such a technique is able to apply to not only menadione, but many other pharmaceutical preparations, which have the capacity of electron donation and acceptance, and the stabilizations of flavin mononucleotide, chlorpromazine, tryptophan, pyridoxine and folic acid etc. are under examination.

Compounds used in this experiments were compound of the Japanese Pharmacopoeia, and used without further purification. The quantitative determination of menadione sodium bisulfite was carried out by ethyl cyanoacetate method,[4] and ultraviolet absorption spectra were measured by Hitachi EPU-2A type photo-electric spectrophotometer, and length of the cell was 10 mm.

Samples were prepared by dissolving menadione and a certain amount of compound to form the complex in *N*/15 phosphate buffer at pH 7.0, and put in a colorless 5 ml. ampule to test the accelaration experiment. Innert gas, such as nitrogen gas, substitution was omitted. The accelaration experiment was carried out by an air thermostat at 50°±1° and two 20 W. fluorescent chemical lamps supplying main wave length of 360 mμ. The sample is put in a distance of 20 cm. from the light source, and temperature rise of the sample was prevented by circulating cooling water of 17~20°.

The authors expresses their sincere thanks to Dr. S. Hayashi, the executive director, and to Dr. U. Imai, director of this laboratory, for permission to publish the report. They are indebted to many coworkers in the laboratory for their kind and helpful advices.

Developing Laboratory of Ukima Factory,
Chugai Pharmaceutical Co., Ltd.,
Ukima-machi Kita-ku Tokyo

Shun-ichi Hata (畑 俊一)
Masao Yamakawa (山川征雄)
Suiichi Tomioka (富岡穂一)
Hajime Sano (佐野 肇)

Received September 10, 1964

4) Shu-yuan Yeh, A. Wiese : Drug Standards, **26**, 22 (1958).

[Chem. Pharm. Bull. 13(1) 99~101 (1965)]

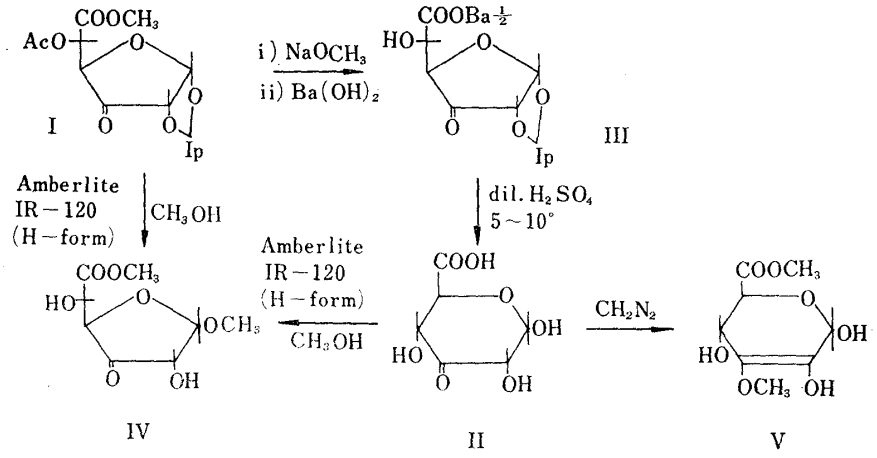
UDC 547.483.07

Synthesis of 3-Keto-D-glucuronic Acid

The preceding paper[1] described the synthesis of methyl 3-keto-5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate (I), which is a key intermediate leading to 3-keto-D-glucuronic acid (II). Attempts to synthesize free 3-keto-aldoses such as II have been unsuccessful.[2] The present communication is concerned with the preparation of II from I and with some observations about structure and chemical properties of II.

Acetyl group of I was removed with 0.1*N* sodium methoxide in dry methanol, and the obtained methyl ester was hydrolyzed with 5% barium hydroxide solution, by which

1) M. Ishidate, Z. Tamura, T. Kinoshita : This Bulletin, **10**, 1258 (1962).
2) O. Theander : "Advances in Carbohydrate Chemistry," **17**, 279 (1962). Academic Press, Inc., New York; Acta Chem. Scand., **17**, 1751 (1963).



barium 3-keto-1,2-O-isopropylidene-D-glucofuranuronate (Ⅲ) was isolated as a yellowish powder. IR ν_{max}^{KBr} cm^{-1} : 1770 (c=0), 1600 (carboxylate), no carbonyl peaks of esters.

Hydrolysis of Ⅲ with dilute sulfuric acid at 5~10° for 5 days gave a hygroscopic crystalline product. After recrystallization from dioxane-acetone (1:2), it showed m.p. 97~98° (decomp.) and mutarotation $[\alpha]_D^{25}$ +50° to −40°. This substance readily reduces ammoniacal silver nitrate solution and neutral potassium permanganate solution at room temperature and gives a single spot of Rf 0.32 on paper chromatography in BuOH-EtOH-H_2O (2:1:1). By reduction with lithium aluminum hydride, this material gave D-allitol as a sole product which was indistinguishable from the authentic sample on electrophoresis in basic lead acetate-buffer.[3] By oxidation with 2.0 moles of periodate, this compound gave *meso*-tartaric acid (ca. 0.27 moles) and with excess of periodate, this compound consumed 2.9 moles of periodate to give 3.0 moles of titrable acid. From these results, the compound should be 3-keto-D-glucopyranuronic acid (Ⅱ). Ⅱ contains dioxane, which was found by means of gas chromatography and nuclear magnetic resonance spectroscopy. (*Anal.* Calcd. for $C_6H_8O_7 \cdot C_4H_8O_2$: C, 42.86; H, 5.74. Found : C, 42.50; H, 5.72.).

Treatment of Ⅱ with Amberlite IR-120 (H form) in methanol afforded methyl (methyl 3-keto-D-glucofuranosid) uronate (Ⅳ), which was also derived from I by the same treatment. Ⅳ reduces ammoniacal silver nitrate solution and neutral potassium permanganate solution at room temperature. (m.p. 119~120° $[\alpha]_D^{25}$ +302° (c=1, H_2O) *Anal.* Calcd. for $C_8H_{12}O_7$: C, 43.64; H, 5.49; Found : C, 43.64; H, 5.60). Furanoside structure of Ⅳ was supported by the fact that Ⅳ consumed 1.0 mole of periodate to form 1.0 mole of titrable acid.

Addition of diazomethane to a methanolic solution of Ⅱ yielded a neutral crystalline product, m.p. 158~160° (decomp.) (*Anal.* Calcd. for $C_8H_{12}O_7$: C, 43.64; H, 5.49. Found : C, 43.50; H, 5.50. IR ν_{max}^{KBr} cm^{-1} : 1754, 1440 (methyl ester), 1623 (C=C), no carbonyl peak on furanoid or pyranoid ring). The product reduces neither ammoniacal silver nitrate solution nor neutral potassium permanganate solution at room temperature and consumes 1.0 mole of periodate to form 1.0 mole of acid and 1.0 mole of formyl ester. Accordingly, the compound was assigned as an enol ether of Ⅱ, methyl (3-O-methyl-D-glucopyranuronate), 2,3-enediol (Ⅴ).

The authors are greatly indebted to Dr. Yoshinobu Hirasaka, Ukima Faculty, Chugai Pharmaceutical Co. Ltd., for many useful suggestions. They wish to thank Chugai Pharmaceutical Co. Ltd., for the supply of the materials. They express their deep gratitude to Dr. Horace S. Isbell, National Bureau of

3) J. L. Frahn, J. A. Mills : Australian J. Chem., **12**, 65 (1959).

Standards, Washington D.C., for his generous gift of D-allitol. Thanks are also due to Dr. Tetsuo Hiraoka, Sankyo Co., Ltd., for the measurement of NMR spectra.

Tokyo Biochemical Research Institute,
Takadaminami-cho,
Toshima-ku, Tokyo, Japan

Morizo Ishidate (石館守三)

Faculy of Pharmaceutical Sciences,
University of Tokyo,
Hongo, Tokyo, Japan

Zenzo Tamura (田村善蔵)
Toshio Kinoshita (木下俊夫)

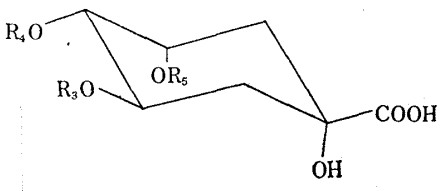
Received September 27, 1964

[Chem. Pharm. Bull. 13(1) 100~104 (1965)]

UDC 547.595.2:582.972

Isolation of 4,5-Di-O-caffeylquinic Acid from Coffee Beans*[1]

In 1950, Barnes, *et al.*[1] isolated "isochlorogenic acid" from coffee beans, but its 5-O-caffeylquinic acid structure[1] has remained in doubt.[2] On the other hand, 4-O-caffeylquinic acid and 5-O-caffeylquinic acid structures, respectively, have since been assigned[3] to neochlorogenic acid (Ⅲ)[4] and "band 510" (Ⅳ)[4] isolated from artichoke leaves and coffee beans. It has further been shown that "isochlorogenic acid" is a mixture of three components.[5,6]



(Ⅰ) quinic acid; $R_3=R_4=R_5=H$
(Ⅱ) chlorogenic acid; R_3=caffeyl, $R_4=R_5=H$
(Ⅲ) neochlorogenic acid; R_5=caffeyl, $R_3=R_4=H$
(Ⅳ) "band 510"; R_4=caffeyl, $R_3=R_5=H$
(Ⅴ) 4,5-di-O-caffeylquinic acid; $R_4=R_5$=caffeyl, $R_3=H$

We have now isolated from unroasted Brazilian coffee beans a white powder to which a 4,5-di-O-caffeylquinic acid structure is assigned. The preliminary isolation and purification procedures were substantially the same as those described for "isochlorogenic acid."[1] The crude acid was further purified by three 50-plate counter-current-distributions between butyl acetate and phosphate buffer (one run at pH 5.7 followed by two runs at pH 4.8); this procedure removed a contaminant with λ_{max}^{MeOH} 233 and 281 mμ as well as some chlorogenic acid (crystalline). In this way an acid, referred to as Compound Ⅰ in this paper, was obtained*[2] with m.p. 140° (decomp.), $[\alpha]_D$ −172° (c=1.0, MeOH); C, 56.31; H, 5.26 (calcd. for $C_{25}H_{24}O_{12}\cdot H_2O$: C, 56.17; H, 4.90); λ_{max}^{MeOH} 330 mμ (log ε

*[1] An outline of this work has been presented at the "Symposium on Recent Developments in Plant Polyphenolics," Delhi, October 1964. Dr. J. Corse and co-workers at Western Research Laboratory, Albany, California, have recently identified "isochlorogenic acid c" (cf. ref. 6) as 4,5-di-O-caffeylquinic acid (private communication).

*[2] The powder was dried at 80° for 20 hours *in vacuo*.

1) H.M. Barnes, J.R. Feldman, W.V. White : J. Am. Chem. Soc., **72**, 4178 (1950).
2) See for example : J. Corse, E. Sondheimer, R. Lundin : Tetrahedron, **18**, 1207 (1962).
3) M.L. Scarpati, P. Esposito : Tetrahedron Letters, No. 18, 1147 (1963).
4) J. Corse : Nature, **172**, 771 (1963).
5) M.L. Scarpati, M. Guiso : Ann. Chem. (Italy), **53**, 1315 (1963); also cf. footnote 1 in reference 3.
6) K.R. Hanson, M. Zucker : J. Biol. Chem., **238**, 1105 (1963).