[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

The Action of Alkali on Alcoholic Solutions of Dihydro Derivatives of Cucurbitacin B

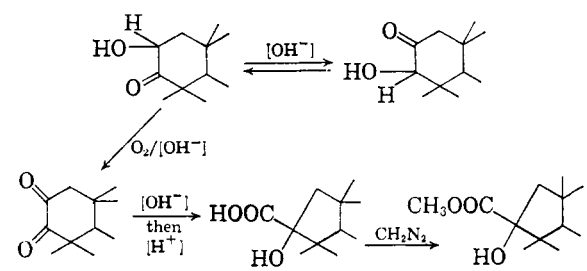
A. MELERA AND C. R. NOLLER

Received May 13, 1960

Dilute methanolic sodium hydroxide catalyzes an isomerization of dihydrocucurbitacin B and of dihydrodeacetoxycucurbitacin B involving the α ketol group in ring A. In ethanolic sodium hydroxide solution the α-ketol group of both compounds is converted by air oxidation to the corresponding diosphenol. The diosphenol from dihydrocucurbitacin B undergoes a further change which appears to involve an internal Claisen ester condensation involving the side chain.

During an investigation of dihydrocucurbitacin B and of dihydrodeacetoxycucurbitacin B,[1] it was observed that they undergo a series of changes in the presence of aqueous methanolic and aqueous ethanolic sodium hydroxide. When dihydrodeacetoxycucurbitacin B, m.p. 204–205°, was dissolved in approximately 0.1*N* aqueous methanolic sodium hydroxide and allowed to stand for four hours, a mixture crystallized from which an isomeric ferric chloride–negative product, m.p. 211–213° was obtained. It was called *isodihydrodeacetoxycucurbitacin B*. If either this product or dihydrodeacetoxycucurbitacin B were dissolved in approximately 0.1*N* aqueous ethanolic sodium hydroxide and the solution were allowed to stand at room temperature for two hours, a ferric chloride–positive product could be isolated, m.p. 173–175°. The ultraviolet absorption spectrum of a solution of this compound in ethanol showed a maximum at 268 mμ, ϵ 8700. When dissolved in 0.02*N* ethanolic sodium hydroxide, the compound absorbed only at 315 mμ, ϵ 6300. This behavior, together with absorption in the infrared at 6.02 μ (1660 cm.$^{-1}$) is characteristic of a diosphenol system.[2] The compound was called *diosphenol-I*. Conversion to the diosphenol could be followed by observing the change in absorption in the ultraviolet which took place rapidly in ethanolic solution and only very slowly in methanolic solution. Acetylation of diosphenol I gave an amorphous enol acetate with λ_{max} 231, ϵ 10,000, thus showing the characteristic hyposchromic shift of 32–36 mμ in going from a 1,2-diketone to an enol acetate.[3] Lavie and Shvo[4] have reported the isolation of diosphenols formed by the autoxidation of alkaline solutions of elatericin A (cucurbitacin D) and of dihydroelatericin A, but no reference is made to the isolation of isomerization products analogous to those reported here from dihydrocucurbitacin B or from dihydrodeacetoxycucurbitacin B.

When dihydrodeacetoxycucurbitacin B was refluxed with 1*N* aqueous ethanolic sodium hydroxide for three hours, about two-thirds was converted to an acidic product which was ferric chloride–negative and gave a crystalline ester on treatment with diazomethane. Analysis indicated that this product is the analog of the methyl ester of ecballic acid.[5] These reactions are explainable by the various formulas proposed for cucurbitacin B[6] all of which have an α ketol grouping in the A ring of a tetracyclic triterpene skeleton. In methanolic solution, hydroxide ion catalyzes the establishment of equilibrium between the two possible α ketol structures, whereas in ethanolic solution the α ketol is oxidized by air to the diosphenol, which undergoes a benzilic acid-type rearrangement when refluxed with aqueous ethanolic sodium hydroxide. These reactions are illustrated by the following partial formulas. It



should be noted that the product obtained by the catalytic hydrogenation of diosphenol-I acetate was not identical with the product of acetylation of dihydrodeacetoxycucurbitacin B[1], and it is assumed that they are diastereoisomers.

Dihydrocucurbitacin B likewise gives an isomeric compound, *isodihydrocucurbitacin B*, with methanolic sodium hydroxide andanot her diosphenol, called *diosphenol-II*, with ethanolic sodium hydroxide. The latter compound in ethanol absorbs at 269 mμ, ϵ 8900, and in 0.02*N* alkali at 315 mμ, ϵ 6200. This diosphenol, however, undergoes a further reaction

(1) W. Schlegel, A. Melera, and C. R. Noller, *J. Org. Chem.*, **26**, 1206 (1961).

(2) E. T. Stiller and O. Rosenheim, *J. Chem. Soc.*, 354 (1938); H. S. French and M. E. T. Holden, *J. Am. Chem. Soc.*, **67**, 1239 (1945); D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 428 (1953); E. Sundt, O. Jeger, and V. Prelog, *Chem. and Ind. (London)*, 1365 (1953).

(3) L. Ruzicka, O. Jeger, and J. Norymberski, *Helv. Chim. Acta.*, **27**, 1185 (1944).

(4) D. Lavie and Y. Shvo, *J. Am. Chem. Soc.*, **81**, 3058 (1959).

(5) D. Lavie, *Chem. and Ind. (London)*, 466 (1956).

(6) D. Lavie and Y. Shvo, *Chem. and Ind. (London)*, 403 (1960); D. Lavie, Y. Shvo, D. Willner, P. R. Enslin, J. M. Hugo, and K. B. Norton, *Chem. and Ind. (London)*, 951 (1959).

on standing in 0.1*N* ethanolic sodium hydroxide. After three days a product was isolated which in ethanolic solution absorbed at 270 mμ, ε 18600 and in 0.02*N* ethanolic sodium hydroxide at 272 mμ, ε 10500 and at 315 mμ, ε 6600. Evidently a new chromophore is present which absorbs at 272 mμ and coalesces with the diosphenol absorption at 268 mμ. In the presence of alkali the absorption at 268 mμ is replaced by the absorption of the diosphenol ion at 315 mμ, whereas the absorption at 272 mμ is not affected. The formation of the new product, which was called *disophenol-III*, can be followed by noting the appearance of the new band at 272 mμ. Analyses of *diosphenol-III* indicate only the loss of one mole of water from diosphenol-II, although the acetoxy band of diosphenol-II at 5.82 μ (1720 cm.$^{-1}$) has disappeared completely. The formation of isodihydrocucurbitacin B and of diosphenol II can be explained in the same way as the formation of isodihydrodeacetoxycucurbitacin B and diosphenol-I. The formation of diosphenol-III may be explained by assuming an internal Claisen ester condensation of the acetate grouping with hydrogen α to a carbonyl group. However, it is difficult to explain the loss of water in the presence of alkali, or even under the neutral or faintly acid condition that may have existed during the isolation.

EXPERIMENTAL

Isodihydrodeacetoxycucurbitacin B. A solution of 250 mg. of dihydrodeacetoxycucurbitacin B[1] in 12.5 cc. of methanol was cooled to 10° and 5 cc. of 0.2*N* aqueous sodium hydroxide added. After standing for 2 hr., 10 cc. of water was added and the mixture warmed to dissolve some amorphous precipitate. After standing 2 hr. longer, 155 mg. of crystals, m.p. 186–194° was obtained. Dilution of the mother liquor with water and extraction with ether gave 90 mg. of amorphous material. A solution of the crystals in the elutant (hexane-benzene, 3 v.: 1 v.) was chromatographed on a Celite-formamide column, collecting 100-cc. fractions. Fractions 9–14 gave a sharp maximum in the chromatogram (97 mg.) and after four crystallizations from acetone-hexane gave 70 mg. of prisms, m.p. 211–213°; $[\alpha]_D^{25}$ 78° (*c* 0.54),[7] infrared 2.92 (OH), 5.87 and 5.91 (C=O). A ferric chloride test was negative.

Anal. Calcd. for $C_{30}H_{46}O_6$: C, 71.68; H, 9.22. Found: C, 71.24, 71.74; H, 8.95, 9.02.

Diosphenol-I. A solution of 1.1 g. of dihydrodeacetoxycucurbitacin B in 1.9 l. of 75% ethanol and 100 cc. of 2*N* aqueous sodium hydroxide was allowed to stand at room temperature for 2 hr. After this time the ultraviolet absorption at 268 mμ had reached a maximum, and the reaction was stopped by neutralizing with acetic acid. The ethanol was removed under reduced pressure at 60° and the residue diluted with water and extracted with ether. The ether solution was washed with water, dried over sodium sulfate, and the ether evaporated to give 1.1 g. of residue which was chromatographed on a 1.5 × 50-cm. Celite-formamide column using hexane-benzene (3 v.: 1 v.) as elutant, and collecting 200-cc. fractions. Fractions 1–4 gave only traces of material. After changing to hexane-benzene (1 v.: 1 v.) fractions 6–9 gave 933 mg. of crystalline product which on recrystallization from acetone-hexane weighed 700 mg., m.p. 173–175°. It gave a deep color with ferric chloride. After three more crystallizations, the analytical sample melted at 174–175°; $[\alpha]_D^{25}$ −62.5° (*c* 1.0); ultraviolet λ_{max} 268 mμ, log ε 3.93; in 0.02 *N* sodium hydroxide, λ_{max} 315 mμ, log ε 3.80; infrared 2.92 (OH), 5.85 and 5.91 (C=O); 6.02 (C=C—C=O).

Anal. Calcd. for $C_{30}H_{44}O_6$: C, 71.97; H, 8.86; O, 19.17. Found: C, 71.88; H, 8.76; O, 19.03.

Further treatment with 0.1*N* ethanolic sodium hydroxide produced no further change as indicated by the ultraviolet absorption spectrum and by the paper chromatogram (compare with the behavior of diosphenol-II).

Acetylation of diosphenol-I with acetic anhydride in pyridine at room temperature gave an amorphous acetate; ultraviolet λ_{max} 231 mμ, log ε 4.0; infrared 2.91 (OH), 5.68 and 8.3 (C=C—OAc), 5.77 and 8.05 (OAc), 5.88 and 5.91 (C=O). When hydrogenated in ethanol using 10% palladium on charcoal as catalyst (Baker and Co.) 1 mole of hydrogen was absorbed to give an amorphous diacetate; $[\alpha]_D^{25}$ −9.8° (*c* 0.97); ultraviolet λ_{max} 282 mμ, log ε 2.41; infrared 2.92 (OH), 5.76 and 8.1 (OAc), 5.80 (OAc), 5.88, and 5.91 (C=O).

Anal. Calcd. for $C_{34}H_{50}O_8$: C, 69.59; H, 8.59; O, 21.82. Found: C, 69.33; H, 8.66; O, 22.20.

This product is not identical with dihydrodeacetoxycucurbitacin B acetate[1] and has been named *dihydrodiosphenol-I acetate.*

Alkaline rearrangement of dihydrodeacetoxycucurbitacin B. A solution of 300 mg. of dihydrodeacetoxycucurbitacin B in 30 cc. of 1*N* aqueous ethanolic (1 v.: 4 v.) sodium hydroxide was refluxed for 3 hr. Dilution with water, extraction with ether, drying, and evaporation of the ether gave 95 mg. of material which reacted strongly with ferric chloride. The aqueous alkaline layer when acidified and extracted with ether gave 200 mg. of acidic material which gave a negative test with ferric chloride. The acid fraction was esterified with ethereal diazomethane and the esters chromatographed on 6 g. of Woelm alumina, activity II, and eluted with benzene-ether (4 v: 1 v.) to give 73 mg. of crystalline material, which after several crystallizations from acetone-hexane melted at 221–222°; $[\alpha]_D^{25}$ −34.0° (*c* 0.646); infrared 2.91 (OH), 5.81 ($COOCH_3$), 5.85 and 5.90 (C=O).

Anal. Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08; OCH_3, 5.82. Found: C, 69.71; H, 9.00; OCH_3, 6.54.

Isodihydrocucurbitacin B. A solution of 500 mg. of dihydrocucurbitacin B[1] in 15 cc. of methanol was cooled to 10° and 10 cc. of 0.2*N* aqueous sodium hydroxide added. After the solution stood for 3 hr. at room temperature, 200 mg. of crystals, m.p. 225–230°, had precipitated. Dilution of the filtrate with water and extraction with ether gave 210 mg. of amorphous material. The chromatograms of both fractions on formamide-impregnated paper, using benzene as the mobile phase, showed them to be a mixture of at least eight compounds with the main spot at R_f 0.879. The combined product was dissolved in benzene with the aid of some ethanol and chromatographed on a 2.6 × 54-cm. Celite-formamide column using benzene as elutant and taking 60-cc. fractions. Fractions 5–7 gave 155 mg. of crystals, m.p. 228–230° dec. After three crystallizations from acetone-hexane it melted at 234–236° and gave a negative ferric chloride test; $[\alpha]_D^{25}$ +54.8° (*c* 0.75); ultraviolet λ_{max} 290 mμ, log ε 2.87; infrared 2.92 (OH), 5.82 and 7.94 (OAc), 5.91 (C=O).

Anal. Calcd. for $C_{32}H_{48}O_8$: C, 68.54; H, 8.63. Found: C, 68.64, 68.75; H, 8.70, 8.51.

Diosphenol-II. To a solution of 600 mg. of dihydrocucurbitacin B in 950 cc. of 95% ethanol was added 50 cc. of 2*N* aqueous sodium hydroxide and the mixture allowed to stand at room temperature. An absorption band at 315 mμ developed very rapidly and reached maximum intensity after 3 hr. The solution was neutralized with acetic acid and the ethanol removed at 60° under reduced pressure until a precipitate began to form. Dilution with water and

(7) Unless otherwise noted all rotations and ultraviolet spectra are in ethanol and infrared spectra in chloroform. All melting points were determined on a Monoscope IV hot stage.

extraction with ether gave 582 mg. of strongly ferric chloride-positive material. The chromatogram on formamide-impregnated paper, using hexane-benzene (1 v.: 1 v.) as mobile phase, showed four spots with those at R_f 0.055 and 0.620 as the main components. The mixture was separated on a Celite-formamide column using hexane-benzene (1 v:1 v.) as elutant and collecting 100-cc. fractions. Fractions 17–20 gave 204 mg. of crystalline material, m.p. 169–170°, which was unchanged on crystallization from ether-hexane; $[\alpha]_D^{25}$ −41.2° (*c* 0.8); ultraviolet λ_{max} 269 mμ, log ε 3.95; in alkaline solution λ_{max} 315 mμ, log ε 3.79; infrared 2.92 (OH), 5.82 (OAc), 5.86 and 5.91 (C=O), 6.00, 6.14 (C=C—C=O); ferric chloride test strongly positive.

Anal. Calcd. for $C_{32}H_{46}O_8$: C, 68.79, H, 8.30; O, 22.91. Found: C, 68.37; H, 8.65; O, 22.72.

Fractions 22–26 eluted with benzene gave 43 mg. of material, m.p. 273–275°. This product was identical with diosphenol-III.

Diosphenol-III. To a solution of 1.1 g. of dihydrocucurbitacin B in 1.9 l. of 95% ethanol was added 100 cc. of 2*N* aqueous sodium hydroxide, and the mixture was allowed to stand for 22 hr. The ultraviolet absorption spectrum showed two strong bands at 272 mμ and 315 mμ. The solution was neutralized with acetic acid, concentrated at 60° under reduced pressure until a precipitate began to form, diluted with water, and extracted with ether. The combined product of three runs weighed 3.1 g. and gave a strong positive test with ferric chloride. It was chromatographed on a 1.5 × 60-cm. Celite-formamide column using hexane-benzene (1 v.:1 v.) as elutant and collecting 200-cc. fractions. Fractions 1–16 gave 370 mg. of amorphous products. Fractions 17–22 were eluted with benzene and gave 1.2 g. which, after crystallization from chloroform-hexane, weighed 700 mg., m.p. 271–273°. The analytical sample was recrystallized from methylene chloride-ether; m.p. 274–275°; $[\alpha]_D^{25}$ +35° (*c* 0.8); ultraviolet λ_{max} 270 mμ, log ε 4.27; in 0.02 *N* ethanolic sodium hydroxide λ_{max} 272 mμ, log ε 4.02 and λ_{max} 315 mμ, log ε 3.82; infrared 2.90 (OH), 5.87 and 5.89, (C=O), 5.99 and 6.13 (C=C—C=O).

Anal. Calcd. for $C_{32}H_{44}O_7$: C, 71.08; H, 8.20; O, 20.72. Found: C, 71.24; H, 8.09; O, 20.81.

A solution of diosphenol-III on further standing in 0.1*N* aqueous ethanolic solution showed no change in absorption in the ultraviolet. On the other hand diosphenol-II under the same conditions soon showed the appearance of a second maximum in the ultraviolet absorption spectrum at 272 mμ, the original diosphenol maximum at 315 mμ remaining unchanged. Isodihydrocucurbitacin B under these conditions after 3 hr. absorbed strongly at 315 mμ and after 22 hr. at both 272 mμ and 315 mμ.

Acknowledgment. This work was supported in part by a research grant, RG 5076, from the National Institutes of Health, U. S. Public Health Service.

STANFORD, CALIF.

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, UNION CARBIDE CHEMICALS CO.]

Improved Synthesis of Scopoletin

DONALD G. CROSBY

Received June 17, 1960

Scopoletin has been prepared in 30% yield from commercial isovanillin. Reaction of isovanillin with peracetic acid provided a good yield of 3-hydroxy-4-methoxyphenyl formate which could be hydrolyzed to 2,4-dihydroxyanisole. Treatment of either the phenol or its formate with ethyl acetoacetate yielded 4-methylscopoletin, while use of the sodium derivative of diethyl oxalacetate gave ethyl scopoletin-4-carboxylate. Hydrolysis, and decarboxylation of the resulting acid, provided the desired coumarin.

Scopoletin (6-methoxy-7-hydroxycoumarin) has been implicated widely in plant processes such as seed germination,[1] growth,[2] differentiation,[3] and disease.[4] The numerous methods reported for its synthesis involve extended series of reactions based on either a preformed coumarin[5] or 2,4-dihydroxyanisole (VI).[6,7] The phenol (VI) has been obtained from various substituted guaiacols through multistep sequences in which maximum yields have remained below 25%.[6-8] Repeated unsuccessful attempts in our laboratory to improve these procedures necessitated development of a more satisfactory route. Reaction of commercial isovanillin (3-hydroxy-4-methoxybenzaldehyde) (I) with a solution of peracetic acid in ethyl acetate provided 3-hydroxy-4-methoxyphenyl formate (II) in 74% yield (Chart I). Saponification of II gave a 72% yield of the desired phenol, while saponification without isolation of the intermediate formate provided a 66% yield of VI based on isovanillin.

Attempts to prepare scopoletin from VI by standard methods such as reaction with sodium ethyl formylacetate or malic acid and sulfuric acid were unsuccessful, although many variations in the reaction conditions were employed. Previous experience in this laboratory had indicated the utility of 85% phosphoric acid as a condensing agent in the Pechmann reaction, and, in this way, a quantitative yield of 4-methylscopoletin (VII) could be obtained easily from II and ethyl aceto-

(1) E. Libbert and H. Luebke, *Flora*, **145**, 256 (1957).
(2) W. A. Andreae, *Nature*, **170**, 83 (1952).
(3) Kathryn Tryon, *Science*, **123**, 590 (1956).
(4) Shirley Andreae and W. A. Andreae, *Can. J. Res.*, **27C**, 15 (1949); L. Reppel, *Planta Med.*, **7**, 206 (1959).
(5) E. Glaser, *Arch. Pharm.*, **266**, 573 (1928); K. Aghoramurthy and T. R. Seshadri, *J. Sci. Ind. Research (India)*, **11B**, 411 (1952); L. Velluz and G. Amiard, *Bull. soc. chim. France*, **1948**, 1109; M. D. Bhavsar and R. D. Desai, *Current Sci. (India)*, **20**, 325 (1951).
(6) F. S. H. Head and A. Robertson, *J. Chem. Soc.*, 1241 (1931).
(7) R. Seka and P. Kallir, *Ber.*, **64B**, 909 (1931); D. N. Chaudhury, R. A. Holland, and A. Robertson, *J. Chem. Soc.*, 1671 (1948).
(8) W. Baker and C. Evans, *J. Chem. Soc.*, 372 (1938).