The chloroform solution of the acid did not change in rotation on standing one week at room temperature. Attempts to recrystallize the acid from hot ethanol caused complete loss of activity.

3-(2-Nitrophenyl)-2,5-dimethyl-4-furoic Acid (III).—This acid was prepared from 10 g. of the iododimethylfuroate and 7 g. of *o*-bromonitrobenzene in the manner previously detailed. There was obtained 2.75 g. of the acid, m.p. 175–176°.

Anal. Calcd. for $C_{13}H_{11}NO_5$: C, 59.77; H, 4.21; N, 5.36. Found: C, 59.86; H, 4.01; N, 5.70.

Resolution of 3-(2-Nitrophenyl)-2,5-dimethyl-4-furoic Acid.—The above acid (2.75 g.) was treated with an equimolar amount of quinine as in the previous cases in dilute alcohol. The solution was evaporated to dryness and the quinine salt was dissolved in 300 ml. of boiling acetone. After cooling for several days, a first crop of crystals (IIIa) was collected. After evaporation of the mother liquor *in vacuo*, a gum was left which solidified upon cooling (IIIb). The salt IIIa, m.p. 167–168°, had $[\alpha]^{25}D$ −38° (*c* 0.1924 g. in 10 ml. of $CHCl_3$). The salt IIIb, m.p. 183–185° had $[\alpha]^{25}D$ −52° (*c* 0.0906 g. in 10 ml. of $CHCl_3$). The salts were recrystallized from ethanol and dried over P_2O_5 at 70° for 24 hours.

Anal. Salt IIIa. Calcd. for $C_{33}H_{35}O_7N_3$: C, 67.69; H, 5.98. Found: C, 68.00; H, 5.87. Salt IIIb. Calcd. for $C_{33}H_{35}O_7N_3 \cdot {}^1/_2H_2O$: C, 66.66; H, 6.06. Found: C, 66.97; H, 6.00.

The rotation of both salts was −65 and −64°, respectively, after recrystallization, indicating racemization during this process.

The acid from salt IIIa had $[\alpha]^{25}D$ −7.8° (*c* 0.1533 g. in 10 ml. of $CHCl_3$) and melted at 170–171° with softening at 162–165°. The acid from salt IIIb had $[\alpha]^{25}D$ 12.3° (*c* 0.1216 g. in 10 ml. of $CHCl_3$) and melted at 168–169° with softening at 156°. The rotation of the chloroform solution fell to zero in three hours of standing at room temperature.

NEW YORK 58, N. Y.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, DEPARTMENT OF SURGERY OF BETH ISRAEL HOSPITAL AND HARVARD MEDICAL SCHOOL]

Synthesis of 2-Naphthyl β-D-Glucopyruronoside and 2-Naphthyl β-D-Glucofururonolactone and their Behavior toward β-D-Glucuronidase[1]

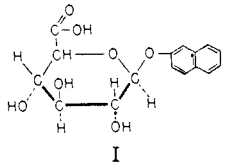
BY KWAN-CHUNG TSOU AND ARNOLD M. SELIGMAN

RECEIVED APRIL 3, 1952

Catalytic oxidation of 2-naphthyl β-D-glucopyranoside with molecular oxygen and platinum black yielded 2-naphthyl β-D-glucopyruronoside. Fusion of 2-naphthol with triacetyl-β-D-glucofururonolactone in the presence of *p*-toluenesulfonic acid yielded 2-naphthyl diacetyl-β-D-glucofururonolactone, which was converted to 2-naphthyl-β-D-glucofururonamide with dry ammonia and methanol. The amide reacted with sodium nitrite in 50% acetic acid to give 2-naphthyl β-D-glucofururonolactone rather than the acid. The pyranoside was readily hydrolyzed by β-D-glucuronidase whereas the furanoside was not. Therefore, substrates susceptible to β-D-glucuronidase activity require a pyranose ring.

In the course of a search for suitable substrates for the colorimetric and histochemical demonstration of β-D-glucuronidase activity,[2] 2-naphthyl β-D-glucopyruronoside (I) and 2-naphthyl β-D-glucofururonolactone (V) were synthesized. Hitherto I has been obtained only by isolation from the urine of man, dog,[3] rabbit[4] and rats[5] after feeding 2-naphthol. The lactone V was not known. Their synthesis and susceptibility to the hydrolytic action of β-D-glucuronidase are given in this report.

Using the procedure of Fernández-García, *et al.*,[6] with some modification, the oxidation of 2-naphthyl β-D-glucopyranoside[7] in aqueous solution with molecular oxygen in the presence of platinum black as catalyst afforded I. The product was isolated through the lead salt and regenerated with hydrogen sulfide. Its infrared spectrum in dioxane showed, in addition to the characteristic 2-naphthyl band pair at 6.11 and 6.21 μ,[7] a carbonyl band at 5.75 μ (Fig. 1) which is in the carboxyl region assigned by the Colthup chart.[8] Its *p*-toluidine salt melted at 186.5–187° (lit. m.p. 184–186°).[5] A mixed melting point of the *p*-toluidine salt of I with a sample prepared from I which we isolated from rabbit urine, showed no depression. Both I and its salt gave a positive naphthoresorcinol test[9] for glucuronide and on hydrolysis in hot 6 *N* sodium hydroxide gave 2-naphthol.



I

The chemical synthesis provides a useful chromogenic substrate for β-D-glucuronidase which should be more practical and more readily available than the tedious biosynthetic preparation of phenolphthalein monoglucuronide currently in use.[10] The latter compound has not been obtained crystalline but is only available as a crude cinchonidine salt.

Moreover, since there could be no change of the oxide ring during the catalytic oxidation of the primary alcohol group of 2-naphthyl β-D-glucopyranoside, the pyranose structure of urinary 2-naphthyl β-D-glucopyruronoside is proved. That a pyranose ring occurs in the natural products has been deduced from methylation study and the periodic acid degradation of bornyl glucuronide[11]

(1) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency, and by an institutional grant to Harvard University from the American Cancer Society.

(2) A. M. Seligman, M. M. Nachlas, L. H. Manheimer, O. M. Friedman and G. Wolf, *Ann. Surg.*, **130**, 333 (1949).

(3) M. Lesnik and M. Nencki, *Ber.*, **19**, 1534 (1886).

(4) F. Bergmann, *Biochem. Z.*, **267**, 296 (1933).

(5) M. Berenbom and L. Young, *Biochem. J.*, **49**, 165 (1951).

(6) R. Fernández-García, L. Amorós, H. Blay, E. Santiago, H. Soltero-Díaz and A. A. Colón, *El Crisol*, **4**, 40 (1950).

(7) K.-C. Tsou and A. M. Seligman, THIS JOURNAL, **74**, 3066 (1952).

(8) N. B. Colthup, *J. Optical Soc. Am.*, **40**, 397 (1950).

(9) B. Tollens, *Ber.*, **41**, 1788 (1908).

(10) P. Talalay, W. H. Fishman and C. Huggins, *J. Biol. Chem.*, **166**, 757 (1946).

(11) J. Pryde and R. T. Williams, *Nature*, **128**, 187 (1931); C. F. Huebner, R. Lohmar, R. J. Dimler, S. Moore and K. P. Link, *J. Biol. Chem.*, **159**, 503 (1945).

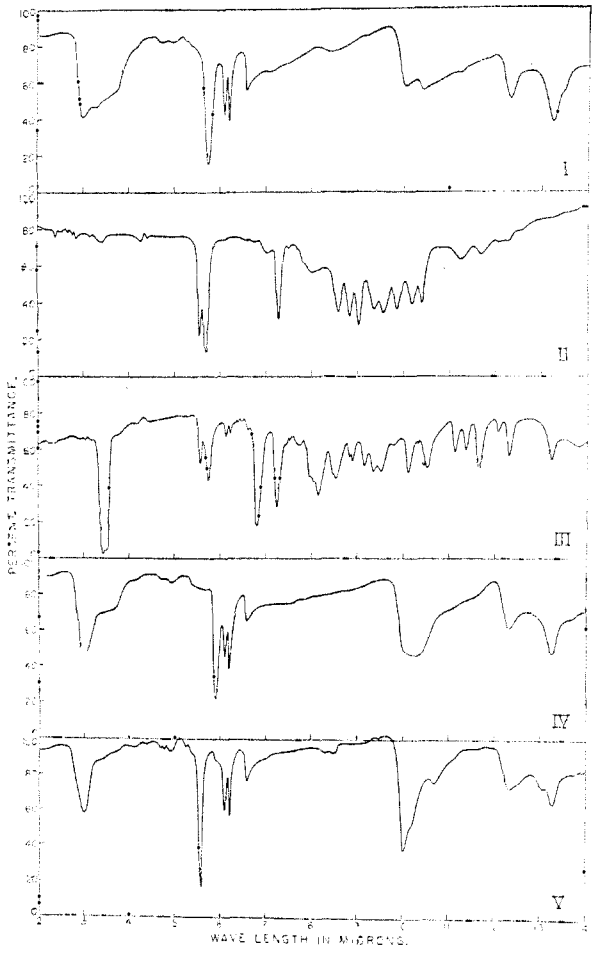
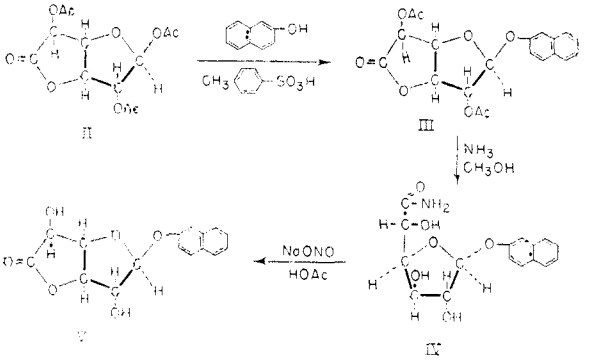


Fig. 1.—Infrared spectra of: 2-naphthyl β-D-glucopyruronoside (I) (in dioxane); triacetyl-β-D-glucofururonolactone (II) (in chloroform); 2-naphthyl diacetyl-β-D-glucofururonolactone (III) (mulled in mineral oil); 2-naphthyl β-D-glucofururonoamide (IV) (in dioxane); 2-naphthyl β-D-glucofururonolactone (V) (in dioxane). These measurements were made with a Baird Infrared Recording Spectrophotometer, Model B. The chloroform was reagent grade and the dioxane was purified by distillation over sodium.

and other glucuronides. Thus, the synthetic evidence supports the contention that naturally conjugated glucuronides are of the pyranoid type.

2-Naphthyl β-D-glucofururonolactone (V) was synthesized as shown in the accompanying scheme. By acetylation of D-glucuronolactone with boron trifluoride as catalyst, triacetyl-β-D-glucofururonolactone (II) was prepared in a better yield than reported by Goebel and Babers[12] who used acetic anhydride and pyridine. Without direct evidence, they tentatively assigned the pyranose structure to II. However, since the change in ring conformation in our scheme is unlikely and since the products proved to be furanosides, we have assigned the furanoid ring to II. The γ-lactone structure was corroborated by a 5.58 μ band in the carbonyl region of the infrared spectrum (Fig. 1). This assignment of structure for II is also in accord with the suggestion put forth by Smith[13] that D-glucuronolactone has two five-member rings. Therefore, we suggest that compounds prepared from II such as acetobromoglucurone,[14] acetochloroglucurone[15] and their derivatives[16] are probably all of the furanoid type.



Fusion of II with 2-naphthol in the presence of *p*-toluenesulfonic acid as catalyst *in vacuo* gave 2-naphthyl diacetyl-β-D-glucofururonolactone (III). The lactone ring of II remained unaffected during the reaction, as shown by the 5.58 μ band in the infrared spectrum of III. Deacetylation of III by a catalytic amount of sodium methoxide or barium oxide could be accomplished only with simultaneous hydrolysis of the glycosidic linkage. Treatment of a suspension of III in dry ammonia at 0° afforded 2-naphthyl β-D-glucofururonamide (IV). The amide gave a positive Weerman test[17] for an α-hydroxy amide, thus establishing the furanoside structure. The infrared spectrum in dioxane showed a carbonyl band at 5.95 μ in the amide region (Fig. 1). Addition of an equivalent amount of sodium nitrite solution to a 50% acetic acid solution of IV yielded 2-naphthyl β-D-glucofururonolactone (V). The lactone structure was assigned by a carbonyl band at 5.58 μ in the infrared spectrum. The β-configuration of V, as well as that of IV and III, was assigned on the basis of optical rotation data. Hydrolysis of the lactone ring of V with dilute alkali even under mild conditions resulted in simultaneous cleavage of the glycosidic linkage with liberation of 2-naphthol. On the other hand, the pyranoside I did not form a lactone in 50% acetic acid or in cold dilute hydrochloric acid. This result could be attributed to the fact that I may exist in the Cl chair conformation. Therefore, for steric reasons, 3,6-cyclization would be difficult (Fig. 2). That a boat form of the pyranose rings of other carbohydrates is unlikely has been suggested by Hassel and Ottar[18] and confirmed by Reeves.[18]

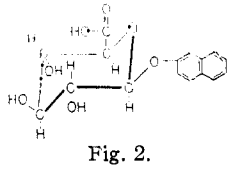


Fig. 2.

(12) W. F. Goebe, and F. H. Babers, *J. Biol. Chem.*, **100**, 743 (1933).
(13) F. Smith, *J. Chem. Soc.*, 584 (1944).
(14) C. Neuberg and W. Neimann, *Z. physiol. Chem.*, **44**, 114 (1905).
(15) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **101**, 173 (1933).
(16) W. F. Goebel and F. H. Babers, *ibid.*, **110**, 707 (1935).
(17) R. A. Weerman, *Rec. trav. chim.*, **37**, 16 (1918); W. N. Haworth, S. Peat and J. Whetstone, *J. Chem. Soc.*, 1975 (1938).
(18) O. Hassel and B. Ottar, *Acta Chim. Scand.*, **1**, 929 (1947); R. E. Reeves, THIS JOURNAL, **72**, 1499 (1950).

Enzymatic hydrolysis by a purified preparation of bovine β-D-glucuronidase[19] and by homogenates of rat spleen which is known to have glucuronidase activity occurred readily at *p*H 4.95 with I, but not at all with V. The requirement of a pyranose ring in substrates susceptible to β-D-glucuronidase activity is thereby demonstrated. Further studies of the specificity requirements of this enzyme are in progress. The method also has been successfully extended to the synthesis of phenyl and 6-bromo-2-napthyl β-D-glucopyruronosides, and will be published later.

Experimental[20,21]

2-Naphthyl β-D-Glucopyruronoside (I).—Oxygen gas was bubbled through an aqueous solution (200 cc.) of 2-naphthyl β-D-glucopyranoside[7] (2 g.) in the presence of 0.5 g. of platinum black and 0.54 g. of sodium bicarbonate on a steam-bath with mechanical vigorous stirring. Oxygen was introduced through two gas bubblers, each fitted with a fritted-glass disc and the sodium bicarbonate was added in two portions at a 30-minute interval. At the end of one hour, the amber colored solution was filtered and cooled. The insoluble glucopyranoside, if any, was removed by filtration and basic lead acetate was added to precipitate the lead salts of the glucopyruronoside. The lead salt was collected and converted to the acid with hydrogen sulfide. Lead sulfide was removed and the filtrate was extracted with ethyl acetate. There was obtained 1.2 g. (55%) of the acid, m.p. 149–150°. The sample was recrystallized from ethyl acetate, m.p. 150–151° (lit. 149–150°),[5] $[\alpha]^{21}D$ −101° (*c* 1.37, ethanol), $[\alpha]^{21}D$ −112° (*c* 2.08, dioxane) (lit. $[\alpha]^{22}D$ −97°, ethanol).[5] Its infrared spectrum in dioxane indicated, in addition to the characteristic 2-naphthyl band pair at 6.11 and 6.21 μ, a carboxyl band at 5.75 μ, and a broad diffuse band in the 3.0–3.8 region, usually characteristic of carboxylic acids.

***p*-Toluidine salt** of I, m.p. 186.5–187° (lit. m.p. 184–186°).[5]

Anal. Calcd. for $C_{23}H_{25}NO_7$ (427.44): C, 64.62; H, 5.90. Found: C, 64.69; H, 6.08.

No depression was observed when the *p*-toluidine salt was mixed with an authentic sample prepared from I isolated from the urine of rabbits fed 2-naphthol.[22]

Both I and its *p*-toluidine salt showed a positive resorcinol test.[9] Hydrolysis of I in either hot 6 *N* HCl on prolonged heating or in 6 *N* NaOH gave 2-naphthol which was demonstrated by coupling with tetrazotized diorthoanisidine. When 0.1 g. of I was dissolved in 5 cc. of 50% acetic acid and allowed to stand at room temperature overnight, it was recovered unchanged. Similarly, when 0.1 g. was suspended in 1 cc. of 6 *N* hydrochloric acid and allowed to stand overnight at 0°, it was recovered unchanged, m.p. 149–150°. Infrared spectra showed no change and no band in the carbonyl region other than the 5.75 μ band was seen. Lactone formation could not be demonstrated.

Brucine salt (dihydrate), m.p. 164° (dec.).

Anal. Calcd. for $C_{39}H_{42}O_{11}N_2 \cdot 2H_2O$ (750.78): C, 62.39; H, 6.18. Found: C, 62.65; H, 5.91.

Triacetyl-β-D-glucofururonolactone (II).—To 17.6 g. of D-glucofururonolactone (m.p. 171–173°)[23] suspended in 40 cc. of acetic anhydride was added in small portions, 4 cc. of boron trifluoride in ether (45%). The resulting dark red solution, after standing at room temperature for one hour, was poured into 200 cc. of water with cooling. The product was collected and recrystallized from ethanol and acetic acid to give 27.0 g. (90%) of II in fine white needles, m.p. 193–194°, $[\alpha]^{20}D$ +89.6° (*c* 2.21, chloroform). There was no depression when it was mixed with a sample prepared by the method of Goebel and Babers.[11] Its infrared spectrum in chloroform gave a γ-lactone band at 5.58 μ and an ester band at 5.78 μ in the carbonyl region (Fig. 1).

2-Naphthyl Diacetyl-β-D-glucofururonolactone (III).—A finely ground mixture of triacetyl-β-D-glucofururonolactone (2.0 g.), 2-naphthol (3.5 g.) and *p*-toluenesulfonic acid (0.1 g.) was fused *in vacuo* for 25 minutes at 100°. The melt was crystallized by addition of ethanol. Washing the precipitate with 95% ethanol and chloroform afforded 2.0 g. (80%) of III in fine clusters, m.p. 227–230°. Recrystallized from methyl cellosolve, pure III melted at 231–232°, $[\alpha]^{22}D$ −4.8 (*c* 1.0, pyridine). Its infrared spectrum in dispersion in mineral oil showed a 5.58 μ band and a 6.10 and 6.20 μ band pair (Fig. 1).

Anal. Calcd. for $C_{20}H_{18}O_8$ (386.34): C, 62.17; H, 4.70. Found: C, 61.91; H, 4.77.

2-Naphthyl β-D-glucofururonamide (IV).—2-Naphthyl diacetyl-β-D-glucofururonolactone (1.8 g.) was suspended in 50 cc. of absolute methanol saturated with dry ammonia at 0°. The reaction mixture was shaken at 4° for three hours. The orange-yellow solution was evaporated to a sirup which was crystallized by addition of 10 cc. of cold water. The precipitate was washed well with ether and recrystallized from 75% ethanol to give 0.35 g. of IV in fine white needles, m.p. 154–155°. Two more recrystallizations gave pure IV which melted at 157–158°; $[\alpha]^{25}D$ −152° (*c* 1.5, dioxane). Its infrared spectrum showed a 5.95 μ band in the carbonyl region which was taken as an evidence that IV was an amide and not an ammonium salt. It gave a positive Weerman test.[17]

Anal. Calcd. for $C_{16}H_{17}NO_6$ (319.30): C, 60.18; H, 5.37; N, 4.39. Found: C, 60.22; H, 5.30; N, 4.65, 4.39.

2-Naphthyl β-D-glucofururonolactone (V).—An equivalent amount of sodium nitrite (56 mg.) was added slowly to 242 mg. of 2-naphthyl β-D-glucofururonamide in 50% acetic acid solution (10 cc.). After four hours, a lemon-yellow solution resulted and evolution of gas had apparently ceased. The solution was evaporated to dryness and 3 cc. of water was added. The precipitate was recrystallized from alcohol and water twice to give 42 mg. of pure V in stout needles which, after drying at 100° over phosphorus pentoxide *in vacuo* for six hours, melted at 185–186° (dec.), $[\alpha]^{23}D$ −115° (*c* 1.10 in dioxane). Its infrared spectrum showed a 5.58 μ band in the carbonyl region (Fig. 1).

Anal. Calcd. for $C_{16}H_{14}O_6$ (302.27): C, 63.57; H, 4.67. Found: C, 63.35; H, 4.97.

Addition of an equivalent amount of water to V in dioxane followed by drying the resulting solution over calcium chloride overnight *in vacuo* yielded a monohydrate, m.p. 178–178.5° (dec.).

Anal. Calcd. for $C_{16}H_{14}O_6 \cdot H_2O$ (320.29): C, 60.00; H, 5.04. Found: C, 60.03; H, 5.07.

The fact that it was not the acid of V was shown by its infrared spectrum in dioxane identical to that of V and by its conversion to V on drying at 100° for six hours over phosphorus pentoxide, *in vacuo*, loss of weight, 5.4%. Treatment of V with dilute sodium hydroxide in an attempt to open the lactone ring resulted in immediate hydrolysis of the glycoside linkage to liberate 2-naphthol.

Enzymatic Hydrolysis.[24]—A commercial preparation of β-glucuronidase[19] (10 mg.) was dissolved in 1 cc. of distilled water and used below. In addition, spleen of a freshly killed rat was homogenized in distilled water (10 mg./cc.) with a motor-driven, ground-glass homogenizer for 2 minutes and was centrifuged for 5 minutes at 2500 r.p.m. The supernatant (1 cc.) or 1 cc. of the commercial preparation was incubated with a solution of each substrate (5 cc.) prepared as follows: Each solution was prepared to contain 1.6×10^{-6} *M* of the substrate dissolved in 4 cc. of water and 1 cc. of phosphocitrate buffer,[25] *p*H 4.95. After 4 hours of incubation at 37°, each tube was alkalinized with 0.2 *M* trisodium phosphate (0.5 cc.), coupled with 1 cc. of tetrazotized diorthoanisidine[26] (1 mg. per cc. dissolved in

(19) Purchased from Viobin Corp., Monticello, Illinois.

(20) All melting points are corrected.

(21) Microanalyses by Mrs. Shirley Golden.

(22) Since it was found that rabbits introduce another hydroxy group into a large fraction of fed naphthol so that the urine contained a hydroxy naphthyl glucopyruronoside as well as I, the urine was treated with an excess of diazotized 1-aminoanthraquinone (followed by treatment with charcoal) in order to couple and render less soluble the hydroxy derivative, prior to isolation of I by precipitation with basic lead acetate.

(23) Provided through the courtesy of Dr. L. W. Smith and the Commercial Solvents Corporation.

(24) These experiments were performed in collaboration with Dr. Selma H. Rutenburg.

(25) R. B. Cohen, K.-C. Tsou, S. H. Rutenburg and A. M. Seligman, *J. Biol. Chem.*, **195**, 239 (1952).

(26) Available in powder form under the trade name du Pont Naphthanil Diazo Blue B.

TABLE I

Substrate	Hydrolysis by purified glucuronidase in 4 hours Color density	Hydrolysis by supernatant rat spleen homogenate in 4 hours Color density
2-Naphthyl β-D-glucopyruronoside	515	900
Brucine salt of 2-naphthyl β-D-glucopyruronoside	540	900
Toluidine salt of 2-naphthyl β-D-glucopyruronoside	240	350
2-Naphthyl β-D-glucofururonolactone	0	0

cold water immediately before use), acidified with 1 cc. of 80% trichloroacetic acid and extracted with chloroform (10 cc.). The color density was measured in a photoelectric colorimeter (Klett) with a 540 mμ filter. The results are given in Table I. A calibration curve for the dye from 2-naphthol is given elsewhere.[27] The 2-naphthyl β-D-glucofururonolactone was not hydrolyzed at all by tissue homogenates or by purified β-glucuronidase. 2-Naphthyl β-D-glucopyruronoside was readily hydrolyzed. The toluidine salt was less readily hydrolyzed than either the acid or brucine salt.

(27) A. M. Seligman and M. M. Nachlas, *J. Clin. Invest.*, **29**, 31 (1950).

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

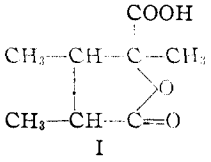
The Structure of Monocrotaline. XIV. Synthesis of Monocrotalic Acid

BY ROGER ADAMS, B. L. VANDUUREN AND B. H. BRAUN

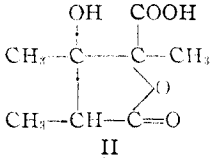
RECEIVED JULY 18, 1952

l-Monocrotalic acid, obtained by the hydrogenolysis of the alkaloid monocrotaline, has been synthesized by the pertungstic acid oxidation of α,β,γ-trimethylglutaconic acid. The levo-isomer of a diastereoisomer of monocrotalic acid was also obtained during resolution of the oxidation product with brucine. The stereochemical configuration of monocrotalic acid is discussed. The previously undescribed 2,3-dimethyl-2,3-dihydroxybutyric acid was synthesized by two methods.

In a previous paper[1] the synthesis of dihydroanhydromonocrotalic acid (I) was described. The structure of monocrotalic acid (II) as deduced by



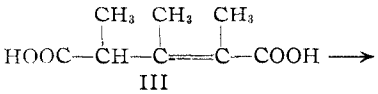
I



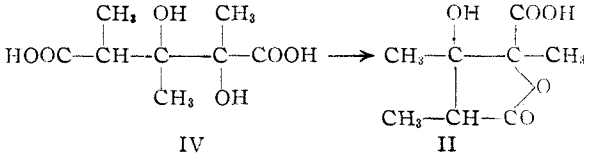
II

degradation reactions[2] was thus confirmed. Attempts to synthesize monocrotalic acid have now been successfully concluded.

The double bond in α,β,γ-trimethylglutaconic acid (III) could not be hydroxylated by means of neutral potassium permanganate; the use of osmium tetroxide and hydrogen peroxide also failed. With pertungstic acid[3] which favors *trans* addition, however, an oily acidic product resulted. This product, which could not be crystallized, proved to be a mixture of diastereoisomers of α,β-dimethyl-β-hydroxy-γ-carboxy-γ-valerolactone (II), formed by lactonization of the α,β,γ-trimethyl-α,β-dihydroxyglutaric acids (IV).



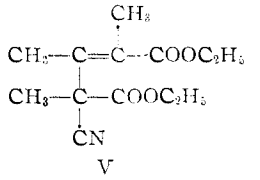
III



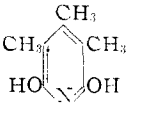
IV II

The oil from the pertungstic acid oxidation was treated with a mole equivalent of brucine in ethanol solution and seeded with the brucine salt of natural monocrotalic acid. An insoluble brucine salt separated which was readily purified and proved to be identical in melting point and rotation with the brucine salt of the authentic natural acid. Hydrolysis of the salt with hydrochloric acid yielded monocrotalic acid, identical in melting point and rotation with monocrotalic acid from monocrotaline.[4] The product gave no depression in melting point on admixture with an authentic specimen of monocrotalic acid. From the mother liquors of the crystallization of the first brucine salt, a second salt was obtained. This salt on hydrolysis yielded an acid, m.p. 180–182°, which gave a depression in melting point on admixture with monocrotalic acid. In a subsequent oxidation and resolution, the salt was not seeded with authentic brucine salt from monocrotalic acid. The brucine salt of the −60.8° acid crystallized out first and the brucine salt of monocrotalic acid was obtained from the mother liquors. From the mother liquors of the crystallization of the first two brucine salts, a third fraction was obtained. This product proved to be a mixture of the salts of the two *d*-rotatory acids and could not be separated by fractional crystallization.

The preparation of trimethylglutaconic acid presented unforeseen difficulties: Diethyl γ-cyano-α,β,γ-trimethylglutaconate (V) was prepared by the previously described method.[5,6] This ester on



V



VI

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