[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF OTTAWA]

3,6-Anhydro-α-D-galactopyranosyl 1,4;3,6-Dianhydro-β-D-fructoside. A Chemical Proof of the Configuration at the Anomeric Center of the Fructose Moiety of Sucrose[1]

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RECEIVED DECEMBER 16, 1957

Treatment of 4,1′,6′-tri-*O*-tosylsucrose pentaacetate with sodium ethoxide was found to yield 3,6-anhydro-α-D-galactopyranosyl 1,4;3,6-dianhydro-β-D-fructoside. Similar treatment of a "sucrose tritosylate" obtained by reaction of sucrose with three moles of tosyl chloride gave a different "trianhydrosucrose." The results complete the proof of the structure of sucrose by purely chemical means.

A "sucrose tritosylate" I was prepared[2,3] by reaction of sucrose in pyridine with three moles of tosyl chloride.[4] Reaction of I with sodium ethoxide in ethanol resulted in the formation of a non-reducing, neutral substance II, m.p. 163–164.5°, $[\alpha]_D$ +117°, with the composition expected for a trianhydrosucrose.

McKeown, Serenius and Hayward[5] recently have reported a crystalline sucrose pentaacetate III which was believed to have the acetyl groups at positions 2, 3, 4, 3′ and 4′.[6] Since the compound II may have arisen from 6,1′,6′-tri-*O*-tosylsucrose,[5] it was of interest to attempt to prepare the compound by way of the tritosylate IV of the pentaacetate III. However, this approach led to the isolation of a second substance V, m.p. 191–192.5°, $[\alpha]_D$ +137.5°, with the properties expected for a trianhydrosucrose. The purpose of this communication is to report a proof of the structure of V and to point out the implications of its formation on the configuration of the anomeric center of the β-D-fructofuranosyl portion of sucrose. The structure of the "trianhydrosucrose" II will be considered in a forthcoming publication.

The non-reducing compound V was extremely sensitive to acid, undergoing rapid hydrolysis in 0.01 *N* hydrochloric acid at room temperature, and the compound was oxidized by periodate at *p*H 5 but not at *p*H 8. The infrared spectrum gave no indication of unsaturation and this inference was supported by the compound's resistance to the periodate–permanganate reagent.[7] The absence of a 1,2-epoxide ring was indicated by the substance's resistance to prolonged treatment both with 25% alkali and with hydrazine at 100°. The ditosylate of V was unaffected by either sodium iodide in acetone or hydrazine at 100°. It could be concluded, therefore, that neither of the two free hydroxyl groups in V were at the 6- or 6′-positions.[2]

Methylation of V gave a dimethyl ether VI which was subjected to acetolysis using sulfuric acid in acetic anhydride. Paper chromatography of the deacetylated product showed the presence of three components (R_f values of 0.29, 0.36 (trace) and 0.61) which were separated by partition chromatography using Celite[8] to hold the static aqueous phase. The infrared spectrum of the compound VII with R_f 0.29 was identical to that of an authentic sample of 2,4-di-*O*-methyl-D-galactose.[9] The identity of VII was further established by a comparison of the properties of its crystalline anilide with that[9] of 2,4-di-*O*-methyl-D-galactose. Since the substance with R_f 0.61 was converted to VII on acetolysis and deacetylation of the product, it must be 2,4-di-*O*-methyl-3,6-anhydro-D-galactose. The substance with R_f 0.36 was not identified.

The isolation of 2,4-di-*O*-methyl-D-galactose (VII) and its 3,6-anhydride establishes V as a trianhydroepisucrose which possesses both the free hydroxyl groups in a 3,6-anhydro-D-galactopyranosyl residue. Since this residue must have been derived from the glucosyl portion of sucrose which is known[10,11] to possess the α-D-configuration, the 3,6-anhydro-D-galactopyranosyl residue must also possess the α-D-configuration. Since the portion of V which was derived from the fructosyl residue of sucrose must possess two anhydro rings, neither of which are of the 1,2-epoxide type, it follows that compound V must be the 3,6-anhydro-α-D-galactopyranosyl 1,4;3,6-dianhydro-β-D-fructoside.

McKeown and co-workers[5] found the methylation of their crystalline sucrose pentaacetate III to introduce methyl groups at the 4-, 1′- and 6′-positions. They suspected that an acetyl group migrated from the 4- to the 6-position during the methylation. It seems probable, however, that III is actually 2,3,6,3′,4′-penta-*O*-acetylsucrose. The fact that it is possible[12] to acylate 1,2,3,4-tetra-*O*-acetyl-β-D-glucose in pyridine (a compound which is known[13] to undergo acetyl group migration with great ease) without migration suggests that the rearrangement would not occur during the tosylation of III. The tritosylate derivative IV underwent the replacement of only one tosyloxy group by iodine when heated with sodium iodide in acetone. In view of the results obtained by McKeown and co-workers,[5] the replaceable tosyloxy group must have been at the 6′-position. The compound IV must therefore be 4,1′,6′-tri-*O*-

(1) This work was conducted as part of Project No. 88 sponsored by the Sugar Research Foundation, New York, N. Y., and is to comprise a portion of a thesis to be submitted by J. P. B. in partial fulfillment of the requirements for the Ph.D. degree.

(2) A. L. Raymond and E. F. Schroeder, U. S. Patent 2,365,776 (1944).

(3) R. C. Hockett and M. Zief, THIS JOURNAL, **72**, 1839 (1950).

(4) The trivial designations "tosyl (Ts)" and "tosylate" refer to "*p*-toluenesulfonyl" and "*p*-toluenesulfonate," respectively.

(5) G. G. McKeown, R. S. E. Serenius and L. D. Hayward, *Can. J. Chem.*, **35**, 28 (1957).

(6) This system for numbering the positions in the sucrose molecule was proposed by Hockett and Zief.[3]

(7) R. U. Lemieux and H. F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

(8) R. U. Lemieux, C. T. Bishop and G. E. Pelletier, *Can. J. Chem.*, **34**, 1365 (1956).

(9) F. Smith, *J. Chem. Soc.*, 1724 (1939).

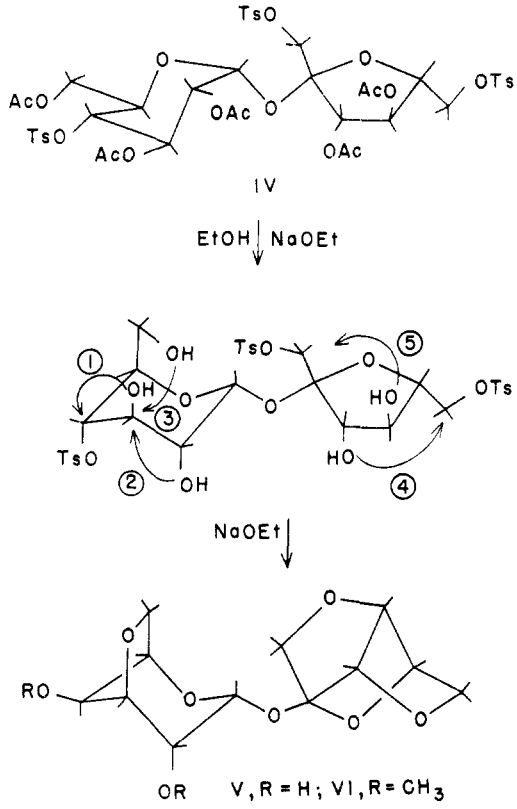
(10) R. U. Lemieux and G. Huber, THIS JOURNAL, **78**, 4117 (1956).

(11) C. A. Beevers and W. Cochran, *Proc. Roy. Soc.* (*London*), **A190**, 257 (1947).

(12) R. U. Lemieux and G. Huber, *Can. J. Chem.*, **31**, 1040 (1953).

(13) B. Helferich and W. Klein, *Ann.*, **455**, 173 (1927).

tosylsucrose pentaacetate. This contention is confirmed by the fact that the formation of the tri-



anhydride V can only be accounted for on the basis of this structure for IV. That is, the formation of the 3,6-anhydro-α-D-galactopyranosyl residue in V would involve the formation of a 3,4-epoxide ring (step 1) followed by migration of the epoxide ring to the 2,3-position (step 2) to make available a route for the closure of the 3,6-anhydro ring (step 3). Thus the *galacto*-configuration is achieved by inversion of C4 (step 1) with the two successive inversions at C3 (steps 2 and 3) leading to a net retention of configuration at this center. Helferich and Müller[14] have prepared a methyl anhydro-β-D-hexoside (VIII), m.p. 158°, $[\alpha]_D$ −118° (water) by the alkaline methanolysis of methyl 4-*O*-tosyl-β-D-glucopyranoside triacetate. The structure of this substance (VIII) has received considerable attention[15,16] and appears to be methyl 3,4-anhydro-β-D-galactopyranoside.[16] Our present results clearly suggest that methyl 3,6-anhydro-β-D-galactopyranoside (IX) should also have been a product of the reaction. Haworth, Jackson and Smith[17] have prepared IX and report it to melt at 119° with $[\alpha]_D$ −115° (water). The dimethyl ethers of VIII and IX are both crystalline compounds with m.p. 83–84°, $[\alpha]_D$ −148.2° (chloroform)[15] and m.p. 83°, $[\alpha]_D$ −87° (chloroform),[17] respectively. Evidently, VIII and IX are not identical. The compound VIII was prepared[14] by extremely mild alkaline alcoholysis of the parent tosyl compound as compared to our conditions for the preparation of V. The compound VIII is being re-examined to test whether or not it can be converted to IX. The steps 4 and 5 proposed for the formation of the 1,4;3,6-dianhydrofructosyl group of V are a well recognized type of reaction. However, the formation of this highly strained structure may not have been anticipated and provides an excellent illustration of the importance of anchimeric assistance in chemical reactions. It is of interest to note that the 1,4;2,5;3,6-trianhydro-D-mannitol recently prepared by Cope and Shen[18] possesses the 1,4;3,6-dianhydrofructofuranosyl residue of V.

The formation of the 1,4;3,6-dianhydrofructosyl group of V can only be accounted for on the basis of the configuration shown in structure IV for the anomeric center of the fructosyl residue. Therefore, the formation of V provides unequivocal chemical proof for the absolute configuration of the anomeric center of the fructosyl moiety of sucrose. The configurational assignment made on this basis is in agreement with that previously established by X-ray crystallographic studies.[11] It does not seem well recognized that the previous chemical and biochemical evidence for the configuration of the fructosyl residue of sucrose[19,20] were ultimately based on the speculation that Hudson's rules of isorotation correlate configuration with rotation in the case of *ketofuranosides* as is now known to be the case for a variety of aldopyranoses and their derivatives.[21] It is of real interest therefore that the present evidence for the structure of sucrose together with that previously obtained by X-ray analysis[11] allow the conclusion that the rules of isorotation in fact do correlate configuration with rotation in the case of fructofuranosides.[22] It can be anticipated therefore that this situation will also apply to other ketofuranosides.

In conclusion, it is noteworthy that the above-described experiments complete the proof of the structure of sucrose by purely chemical means.

Acknowledgments.—The authors wish to thank Dr. C. T. Bishop for the authentic samples of 2,4-di-*O*-methyl-D-galactose and its anilide.

Experimental[23]

"Trianhydrosucrose" II.—Dry sucrose (80 g., 0.224 mole) was dissolved in 1600 ml. of boiling pyridine (dried over phosphorus pentoxide). Tosyl chloride (128 g., 0.672 mole) was added to the solution cooled to 0° and the resulting solution was stored at 5° for one week. The pyridine was evaporated *in vacuo* and the sirupy residue was dissolved in chloroform. The chloroform solution was washed first with ice-cold 0.5 *N* hydrochloric acid, then with aqueous sodium bicarbonate solution and, finally, with water. After drying over sodium sulfate, the chloroform was removed *in vacuo* to leave a white amorphous residue (I) which possessed a sulfur content 11.1%, near that expected (11.9%) for a sucrose tritosylate.[2,3] The yield was near quantitative (97%). The material I, 175 g., was dis-

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(15) A. Müller, *ibid.*, **67B**, 421 (1934); **68B**, 1094 (1935).
(16) A. Müller, M. Moricz and G. Verner, *ibid.*, **72B**, 745 (1939).
(17) W. N. Haworth, J. Jackson and F. Smith, *J. Chem. Soc.*, 620 (1940).
(18) A. C. Cope and T. Y. Shen, THIS JOURNAL, **78**, 5912 (1956).
(19) I. Levi and C. B. Purves, *Adv. Carbohydrate Chem.*, **4**, 27 (1949).
(20) M. L. Wolfrom and F. Shafizadeh, *J. Org. Chem.*, **21**, 88 (1956).
(21) R. U. Lemieux, *Can. J. Chem.*, **29**, 1079 (1951).
(22) C. B. Purves and C. S. Hudson, THIS JOURNAL, **59**, 49 (1937).
(23) All melting points are uncorrected. The rotations were measured at room temperature, 20–25°, using the D-line of sodium. The microanalyses for carbon and hydrogen were made by the Geller Laboratories, West Englewood, N. J.

solved in one liter of *N* sodium ethoxide in ethanol and the solution was refluxed for one hour. The residue, obtained on evaporation of the ethanol *in vacuo*, was mixed with 750 ml. of water and 250 ml. of chloroform. The aqueous layer was extracted with 50 ml. of chloroform which, after washing with an equal volume of water, was combined with the main chloroform extract. Evaporation of the chloroform gave 22 g. of a dark brown sirupy product the composition of which has not been investigated. The combined aqueous extracts were extracted continuously with ether for two weeks. Evaporation of the ether extract left a residue which on crystallization from ethanol gave 1.78 g. (3.5% over-all yield) of crude "trianhydrosucrose" II, m.p. 158–160°. Two further recrystallizations from ethanol afforded the pure compound, m.p. 163–164.5°, $[\alpha]_D$ +117° (*c* 0.9 in water).

Anal. Calcd. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.73; H, 5.75.

The diacetate of II was prepared by heating the compound with acetic anhydride and sodium acetate. The compound, m.p. 181.5–182.5°, $[\alpha]_D$ +128.6° (*c* 1.8 in chloroform) was isolated in the usual manner and purified by recrystallization from ethanol.

Anal. Calcd. for $C_{16}H_{20}O_{10}$: C, 51.61; H, 5.41; sapn. equiv., 186.2. Found: C, 51.53; H, 5.34; sapn. equiv., 185.

4,1′,6′-Tri-*O*-tosylsucrose Pentaacetate (IV).—The crystalline sucrose pentaacetate III, m.p. 155–156°, $[\alpha]_D$ +22° (*c* 3.1 in chloroform),[5] was treated with a twofold excess of tosyl chloride in pyridine for one week at 5°. After this time, the excess tosyl chloride was decomposed by the addition of a little water and the solution was poured as a fine stream into a large excess of cold water. The amorphous white precipitate was washed first with 2% hydrochloric acid and finally with water. The yield of dry amorphous material IV, m.p. 85–91°, was 91%.

Anal. Calcd. for $C_{43}H_{50}O_{22}S_3$: S, 9.45. Found: S, 9.12.

The substance IV, 400 mg., was heated at 100° for 17 hours with 1 g. of sodium iodide in 25 ml. of acetone. The amorphous product, 378 mg. (89% yield), isolated in the usual manner, possessed an iodine content, 12.92%, near that (12.81%) expected for monoiodomonodeoxysucrose ditosylate pentaacetate. In contrast to this result, acetylation of the "sucrose tritosylate" I gave a "sucrose tritosylate pentaacetate" which on treatment with sodium iodide in acetone was converted to a substance containing 23.4% iodine. The iodine content calculated for diiododideoxysucrose monotosylate pentaacetate is 26.54%.

3,6-Anhydro-α-D-galactosyl 1,4;3,6-Dianhydro-β-D-fructoside (V).—The sucrose tritosylate pentaacetate IV, 15 g., was added to 200 ml. of *N* sodium ethoxide in ethanol. The solution was refluxed for two hours and the ethanol removed by distillation *in vacuo*. Examination of the residue by paper chromatography revealed that the tosylate IV was converted in high yield to a substance with R_f 0.36 (1-butanol–water system). Only trace amounts of two other products with higher R_f values could be detected. The residue was dissolved in water and the dark-brown solution was extracted continuously with methyl ethyl ketone for one week. Evaporation of the solvent left 1.5 g. of a residue which proved to contain sodium tosylate. The substance was consequently deionized by percolating an aqueous solution first through a bed of a quaternary ammonium type resin (Dowex 1-X10) and then through a bed of carboxylic acid type resin (Amberlite IRC-50-H). Evaporation of the water *in vacuo* left a crystalline residue which was recrystallized from ethanol. The yield of pure material V, m.p. 191–192.5°, $[\alpha]_D$ +137.5° (*c* 1.6 in water), was 0.86 g. The infrared spectrum differed markedly from that of the "trianhydrosucrose" II both when determined as Nujol mulls and in potassium bromide windows.

Anal. Calcd. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 50.00; H, 5.71.

The diacetate of V, m.p. 137.5–138.5°, $[\alpha]_D$ +94.3° (*c* 2 in chloroform), was prepared as described above.

Anal. Calcd. for $C_{16}H_{20}O_{10}$: C, 51.51; H, 5.41; sapn. equiv., 186.2. Found: C, 51.42; H, 5.50; sapn. equiv., 185.

Some Chemical Properties of V.—The compound V was heated at 100° in 25% aqueous potassium hydroxide for 24 hours. The solution remained colorless and chromatography of a sample of the solution on paper showed V to have remained unchanged.

When V was dissolved in hydrazine at 100° and the solution was kept at this temperature for 12 hours, on cooling, the substance crystallized unchanged.

The compound V was dissolved in 0.01 *N* hydrochloric acid and the solution was kept at 23°. Aliquots were removed at various times, neutralized with sodium hydroxide solution, and the neutral solution applied to paper for chromatography using 1-butanol–water as solvent. The substance was extensively degraded to materials which appeared as a streak, R_f 0.2–0.75, after 5 min. reaction time.

Compound V was unaffected by Fehling solution and was completely resistant to periodate at *p*H 8 and room temperature for 3 hours. However, it did not survive the treatment with periodate at *p*H 5. The substance, spotted on paper, was highly resistant to the periodate–permanganate spray reagent of Lemieux and Bauer.[7]

The ditosylate of V, m.p. 158–159° (calcd.: S, 10.75. Found: S, 10.95) was prepared in the usual manner. The compound was unchanged both by sodium iodide in acetone at 100° and by hydrazine at 100°. Methyl 2,4-di-*O*-mesyl-3,6-anhydro-α-D-galactoside has been shown[24] to be resistant to sodium iodide.

Di-*O*-methyl 3,6-Anhydro-α-D-galactosyl 1,4;3,6-Dianhydro-β-D-fructoside (VI).—The compound V, 200 mg., was added to 10 ml. of methyl iodide containing 0.5 g. of silver oxide. The mixture was heated under reflux with mechanical stirring. After 15 minutes a second 0.5 g. of silver oxide was added and this procedure was continued until a total of 2 g. of silver oxide was added. After refluxing overnight, the silver oxide was collected by filtration and washed several times with boiling ethanol. The combined filtrates were evaporated and the residue was decolorized with charcoal and recrystallized from ethanol. The yield was 150 mg., m.p. 105–106°, $[\alpha]_D$ +48.6 (*c* 1.45 in chloroform).

Anal. Calcd. for $C_{14}H_{20}O_8$: methoxyl, 19.62. Found: methoxyl, 19.5, 19.7.

2,4-Di-*O*-methyl-D-galactose (VII).—The dimethyl ether VI, 50 mg., was dissolved in 1 ml. of acetic anhydride and 0.03 ml. of concentrated sulfuric acid was added. The solution was stored at 5° for 24 hours, during which time it developed a dark-green coloration. The solution was poured into an ice–water mixture to decompose the excess acetic anhydride and the aqueous mixture was extracted with chloroform. The chloroform solution was freed of acetic acid by shaking with aqueous sodium bicarbonate solution and dried over sodium sulfate. Removal of the chloroform left a sirupy residue, 45 mg., which was taken up in 50 ml. of hot water for deacetylation by passing the solution through a column (45 × 8 mm.) of strongly basic resin (Dowex 1-X10) and elution with 400 ml. of water. Evaporation of the water *in vacuo* gave a sirup which showed components with R_f values of 0.29, 0.36 (trace) and 0.61 when chromatographed on Whatman No. 1 paper using 1-butanol saturated with water for development and the aniline phthalate spray reagent[25] to detect the positions of the materials on the chromatograms. The mixture, 28 mg., was added to the top of a Celite column (500 × 20 mm.) for partition chromatography using the 1-butanol–water system following the procedure of Lemieux, Pelletier and Bishop.[8] The effluent was collected in successive 5-ml. fractions and the component with R_f 0.61 was detected in fractions 19 to 23 and that with R_f 0.29 in fractions 37 to 43. The appropriate fractions were combined and reduced to dryness *in vacuo*. In each case, the residue crystallized. The substance, R_f 0.29, 10 mg. yield, possessed an infrared spectrum identical in every detail to that of an authentic sample of 2,4-di-*O*-methyl-D-galactose.[9] The compounds in each case were mounted in potassium bromide windows using the technique of Lemieux, Epp and Bauer.[26] The identity of the material was confirmed by preparation of the anilide derivative, m.p. 214.5–216°,

(24) A. B. Foster, W. G. Overend, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 2542 (1949).

(25) S. M. Partridge, *Nature*, **164**, 443 (1949).

(26) R. U. Lemieux, A. Epp and H. F. Bauer, Abstracts of Papers, Fall Meeting of the American Chemical Society, Sept. 12–16, 1955, Minneapolis, Minn.

$[\alpha]_D$ −162° (*c* 0.1 in pyridine)[27] the melting point of which was unchanged by admixture of an authentic sample of the anilide[9] of 2,4-di-*O*-methyl-D-galactose, m.p. 215.5–216°, $[\alpha]_D$ −183° (pyridine). The infrared spectra (Nujol mulls) of the two anilides were identical.

The component, R_f 0.61, 15 mg. yield, undoubtedly was 2,4-di-*O*-methyl-3,6-anhydro-D-galactose which survived the acetolysis since the compound was converted to 2,4-di-*O*-methyl-D-galactose (identified by paper chromatography) by the procedure described above for the acetolysis of VI and deacetylation of the product.

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(27) An error of at least 15% may have been involved in measuring this rotation and, consequently, its divergence from that reported for the compound is not surprising. The rotation serves to show, however, that the acetolysis of VI did not yield 2,4-di-*O*-methyl-DL-galactose as may have been anticipated from the results obtained by T. L. Cottrell and E. G. V. Percival [*J. Chem. Soc.*, 749 (1942)] on the acetolysis of methyl 3,6-anhydro-β-D-galactopyranoside.

[CONTRIBUTION FROM THE CHEMISTRY RESEARCH LABORATORY, DEPARTMENT OF SURGERY, UNIVERSITY OF WASHINGTON]

Derivatives of Fluorene. III. Stereoisomerism and Polymorphism of N-Aryl Azomethines[1]

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RECEIVED JULY 15, 1957

A pair of azomethine stereoisomers and a pair of enantiotropic polymorphs in the 9-phenyliminofluorene series have been prepared, isolated and characterized.

Introduction

During the preparation of a series of azomethine derivatives of fluorene, it was found that 2-nitrofluorenone condensed with *p*-toluidine in the presence of a Lewis acid catalyst to give a product isomeric with the product obtained from the condensation of 2-nitrofluorene with *p*-nitrosotoluene in the presence of a base. The melting points of the two compounds differed by 26°. Analogous reactions substituting *p*-nitrosoethylbenzene and *p*-aminoethylbenzene for *p*-nitrosotoluene and *p*-toluidine, respectively, yielded the nitrone in one case and the anil in the other.

In a similar series, the condensation of 2,5-dinitrofluorenone with *p*-fluoroaniline in the presence of a Lewis acid yielded two products,[2] large hexagonal red-colored plates and fine yellow needles. Both had the same elemental analysis and molecular weight. The melting points differed by about 2°. When the catalyst was zinc chloride the main product was the red solid. With aluminum chloride the product was an equal mixture of both forms. A mixture melting point was not depressed. These substances undoubtedly are polymorphs.

While several reports of the isolation of stereoisomeric azomethines have appeared in the literature, they are in question or have been proved polymorphs rather than isomers.[3-6] On the basis of dipole moment measurements, it has been suggested that phenylimino Schiff bases can exist in the *trans* form only,[4] and, in the case of benzylideneaniline, a linear arrangement for the N-phenyl group has been suggested.[7] Thus it was of interest to investigate the compounds we obtained because of the question concerning the ability of an azomethine to exist in two stereoisomeric forms.

Experimental[8,9]

N-(2-Nitrofluorenylidene)-*p*-toluidine. Method—A. A mixture of 5.6 g. (0.025 mole) of 2-nitrofluorenone (m.p. 224.5–225.0°), 4.3. g. (0.040 mole) of *p*-toluidine and 0.25 g. of freshly fused zinc chloride was heated at 160–170° for 45 min.; a melt quickly formed. The cooled reaction mixture was dissolved in hot chloroform and the solution was filtered. The insoluble toluidine–zinc chloride compound was removed by filtration. A mixture of amorphous yellow unreacted 2-nitrofluorenone and crystalline orange-colored product totaling 7.4 g. was obtained by crystallization from the chloroform. The density of the product was much greater than that of the 2-nitrofluorenone; thus the latter was removed by stirring the mixture in ether and decanting. The procedure was repeated until a nearly pure product remained. It then was recrystallized from chloroform, heated under vacuum at 184° until a sublimate was no longer evident and recrystallized twice from benzene; yield 5.6 g. of large, orange-colored rods, m.p. 192–193°. Crystallization from acetone gave m.p. 192–193°.

Anal. Calcd. for $C_{20}H_{14}N_2O_2$: C, 76.42; H, 4.49; N, 8.91; mol. wt., 314. Found: C, 76.22; H, 4.31; N, 8.83; mol. wt. (Rast method), 311.

Method B.—Five drops of 1% sodium ethoxide in ethanol was added to a refluxing solution of 4.2 g. (0.020 mole) of 2-nitrofluorene (m.p. 159.0–159.5°) and 3.4 g. (0.028 mole) of *p*-nitrosotoluene (m.p. 47–48°)[10] in 450 ml. of absolute ethanol. The solution was refluxed 3 hours. The product crystallized from the cooled solution and was collected on the filter. Further crops were obtained by concentrating the mother liquors: yield 6.08 g., m.p. 210–215°. Crystallization from benzene raised the m.p. to 214.5–215.5°. Crystallization from acetone produced orange-colored rods, m.p. 217.5–218.5°. A series of mixture melting points of 20%, 40%, 50%, 60% and 80% of the product from A in that from B were 193–207°, 192–202°, 189–203°, 190–202° and 190–197°, respectively.

Anal. Calcd. for $C_{20}H_{14}N_2O_2$: C, 76.42; H, 4.49; N, 8.91; mol. wt., 314. Found: C, 76.27; H, 4.46; N, 8.96; mol. wt. (Rast method), 307.

Inoculation of a supersaturated solution of the product from method A in benzene with a crystal of the product from method B produced no crystallization at room temperature for three days, although the crystal used as seed did not

(1) This work was supported in part by a research grant (C-1744) from the National Cancer Institute of the National Institutes of Health, Public Health Service. For the preceding paper in this series see footnote 2.

(2) M. E. Taylor and T. L. Fletcher, *J. Org. Chem.*, **21**, 523 (1956).

(3) O. Anselmino, *Ber.*, **40**, 3465 (1907).

(4) V. De Gaouck and R. J. W. Le Fèvre, *J. Chem. Soc.*, 741 (1938).

(5) W. Manchot and J. R. Furlong, *Ber.*, **42**, 3030 (1909).

(6) V. De Gaouck and R. J. W. Le Fèvre, *J. Chem. Soc.*, 1392 (1939).

(7) C. Wiegand and E. Merkel, *Ann.*, **550**, 175 (1942).

(8) All melting points are corrected.

(9) Analyses were performed by Drs. Weiler and Strauss, Oxford, England, and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(10) Prepared by method of R. Lutz and M. Lytton, *J. Org. Chem.*, **2**, 73 (1937).