graphically pure esculetin (300 mg.) was used as the reference esculetin, m.p. 270°, with decomposition.[7]

*Esculetin in tobacco leaves and in cigarette tobacco.* For identification of the relatively smaller amount of esculetin present in tobacco leaves, the procedure described above for tobacco flowers was used. In addition, for some samples, a paper chromatographic procedure was employed which did not involve the preliminary silicic acid chromatography. The first steps of this procedure were the same through the development with the nitromethane-benzene-water system as those already described by Yang *et al.*[2] for the quantitative determination of scopoletin in cigarette tobacco. With the nitromethane system, the scopoletin ($R_f$ = 0.84) moved far ahead of the esculetin ($R_f$ = 0.07). This time, the esculetin zone, still containing another interfering blue fluorescent compound, was cut out and eluted with methyl alcohol. The eluates were streaked on new sheets of S & S paper and developed in 15% acetic acid–water, and then again in the nitromethane system. Each section containing the esculetin was cut out, sewn on a new sheet, developed in the ethyl acetate–formic acid–water system to move the esculetin across the sewing line, and the paper removed and dried. The unfinished chromatogram was then developed again in 15% acetic acid–water to effect the separation of esculetin from the other blue fluorescent compound. Usually esculetin moved sufficiently ahead of the interfering substance at this point to be eluted with methyl alcohol as a chromatographically pure compound and then be identified beyond doubt as esculetin. If not completely separated, the esculetin zone was placed on yet another paper and rechromatographed in the 15% acetic acid–water before making further identification studies.

By one or both of the above procedures, esculetin was identified as being present in a small amount in leaves of Burley tobacco (Kentucky 16), Turkish tobacco (imported and domestic), and flue-cured tobacco (Hicks) from North Carolina.

Because of the low amount of esculetin present relative to that of scopoletin in cigarette tobacco, 8 g. samples were used for these analyses instead of the 2 g. samples used for analysis of scopoletin. Also, Whatman No. 3 MM chromatography paper was used for the first step only in the paper chromatography. The S & S No. 589 red ribbon paper was used for the other paper chromatographic steps. Cigarettes analyzed included Camel, Lucky Strike, Philip Morris, Old Gold Straights, Pall Mall, Viceroy, Winston, and Oasis.

*Esculetin in the mainstream smoke from cigarettes.* The sampling and smoking of 8 brands of cigarettes for esculetin analysis were similar to those already described for scopoletin by Yang *et al.*[2] The separation, purification, and identification of esculetin from the cigarette smoke condensates were carried out by mass paper chromatography in the same manner described above for esculetin in tobacco leaves. Because esculetin was present only in trace amounts in the smoke, eluates representing smoke from 4 packs of cigarettes had to be combined and concentrated to obtain sufficient esculetin for unambiguous chromatographic studies.

*Acknowledgment.* This work and some previous research on which these findings are based were supported in part by the Tobacco Industry Research Committee and by the Atomic Energy Commission.

We also sincerely thank Dr. Norbert Scully and Mr. Will Chorney of the Argonne National Laboratory, Dr. C. W. Nystrom, R. J. Reynolds Tobacco Co., Dr. J. M. Moseley, American Tobacco Co., and Luther Shaw, Waynesville, N. C., for supplying various tobacco fractions used.

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(7) Handbook of Chemistry and Physics, 38th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1956.

## Addition of Dinitrogen Pentoxide to Stilbene

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*Received January 29, 1959*

The reaction of dinitrogen pentoxide and simple olefins to produce 1,2-nitronitrates has been shown to be a *cis* addition process.[1] As part of this study the reaction of dinitrogen pentoxide and *cis*- and *trans*-stilbene has been investigated. Recently, the *cis* addition of acetyl nitrate to *trans*-stilbene was reported and DL-*threo*-α-acetoxy-α′-nitrobibenzyl was characterized.[2] This compound was a key intermediate in the proof of configuration of the expected products of the dinitrogen pentoxide-stilbene reaction, the α-nitrato-α′-nitrobibenzyls.

Addition of dinitrogen pentoxide to *trans*-stilbene in the presence of tetraethylammonium nitrate[1] produced a mixture of α-nitrato-α′-nitrobibenzyls (81% yield) which was separated into compounds melting at 96° and 165°. The 96° isomer was the predominant product; quantitative infrared analysis of the mixture isolated indicated that it comprised at least 81% of this mixture. Assignment of the *threo* configuration to this α-nitrato-α′-nitrobibenzyl, m.p. 96°, was made on the basis that the same α-hydroxy-α′-nitrobibenzyl[3] which produced the DL-*threo*-α-acetoxy-α′-nitrobibenzyl, m.p. 135°,[2] was converted to the nitronitrate of m.p. 96° on nitration with dinitrogen pentoxide. Thus, the addition of dinitrogen pentoxide to *trans*-stilbene was predominantly a *cis* process.

When the addition of dinitrogen pentoxide to *cis*-stilbene was attempted under the conditions of the *trans*-stilbene addition little reaction occurred and most of the stilbene was recovered.[4] Increasing the reaction time led to a higher yield of nitrated products, but considerable ring nitration apparently occurred. However, the DL-*erythro*-α-nitrato-α′-nitrobibenzyl, isolated in 8.6% yield, comprised a

(1) T. E. Stevens and W. D. Emmons, *J. Am. Chem. Soc.*, **79**, 6008 (1957).

(2) G. Drefahl and H. Crahmer, *Ber.*, **91**, 745 (1958).

(3) The *threo*-α-hydroxy-α-nitrobibenzyl, m.p. 106°, was obtained from the stilbene–dinitrogen tetroxide reaction, the details of which will be reported later. DL-*Erythro*-α-hydroxy-α′-nitrobibenzyl, m.p. 99°, also was obtained from this reaction and was converted to DL-*erythro*-α-acetoxy-α′-nitrobibenzyl, m.p. 116°.

(4) The recovered stilbene was mainly the *trans* form, but isomerization undoubtedly took place during the work-up of the reaction mixture. In the reaction of acetyl nitrate and *cis*-stilbene isomerization proceeded faster than addition.[2]

larger percentage of the nitronitrate fraction than it did in the *trans*-stilbene experiment, indicating that the *cis* addition process, though slow, was operative.

## EXPERIMENTAL[5]

*Reaction of trans-stilbene and dinitrogen pentoxide.* A stirred solution of 3.0 g. (16.6 mmoles.) of stilbene and 3.5 g. (18 mmoles.) of tetraethylammonium nitrate in 100 ml. of methylene chloride was cooled to −20° while 16.6 mmoles. of dinitrogen pentoxide in 18.5 ml. of methylene chloride was added over 15 min. After addition of the dinitrogen pentoxide the mixture was stirred at −5° for 15 min. and at 3° for 45 min. Water (100 ml.) was then added to the reaction mixture and the organic layer was separated and washed with aqueous sodium bicarbonate and water and dried over magnesium sulfate. Removal of the methylene chloride left 4.0 g. of residue. The residue was taken up in methylene chloride and chromatographed on a 2.8 × 40 cm. silica gel column. Elution of the column with ligroin–methylene chloride 1:1 gave a fraction which after trituration with ligroin consisted of 3.84 g. (81%) of mixed *threo-* and *erythro-*α-nitrato-α′-nitrobibenzyls, m.p. 74–78°. A 2.00-g. portion of this mixture was recrystallized from ligroin four times to give *erythro*-α-nitrato-α′-nitrobibenzyl, 0.07 g., m.p. 157–160°. Further recrystallization from ligroin raised the m.p. to 165–166° dec.

*Anal.* Calcd. for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.80; H, 4.24; N, 9.28.

The ligroin filtrate[6] on standing deposited *threo*-α-nitrato-α′-nitrobibenzyl as a cluster of needles, m.p. 95.5–96.5°. Three recrystallizations from ligroin gave long needles, m.p. 96–97°.

*Anal.* Calcd. for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.67; H, 4.77; N, 9.65.

The amount of *threo* isomer present in the mixture isolated was determined by quantitative infrared analysis using dimethyl sulfoxide as solvent and the 11.37 μ band present only in the *threo* compound as a reference. The sample was found to be 81% the *threo* isomer; the remainder was assumed to be the *erythro*-nitronitrate.

*Reaction of cis-stilbene and dinitrogen pentoxide.* The procedure outlined above for the nitration of *trans*-stilbene was followed using 3.0 g. of *cis*-stilbene. The nitronitrate fraction isolated after chromatography weighed 0.51 g. When the nitration was allowed to proceed for 2 hr. at 3° and the residue handled as usual, there was obtained from the chromatographic column *trans*-stilbene, 0.88 g. (29%), identified by m.p. and infrared spectrum and a nitronitrate fraction of 1.13 g. Two recrystallizations of this material from ligroin gave DL-*erythro*-α-nitrato-α′-nitrobibenzyl, 0.41 g., 8.6%, m.p. 159–162°.

*Nitration of threo-α-hydroxy-α′-nitrobibenzyl.* A stirred solution of 0.78 g. (3.2 mmoles.) of *threo*-α-hydroxy-α′-nitrobibenzyl and 2.0 g. of tetraethylammonium nitrate in 50 ml. of methylene chloride was cooled to −20° while 3.6 mmoles. of dinitrogen pentoxide in 4 ml. of methylene chloride was added dropwise. After addition of the dinitrogen pentoxide the solution was allowed to warm to 0° over 1 hr. The organic layer was then washed with water, aqueous sodium bicarbonate, and water, and was dried over magnesium sulfate. The methylene chloride solution was concentrated to 25 ml. and then chromatographed on a 2.5 × 12 cm. silica gel column. The material eluted by 150 ml. of methylene chloride, 0.89 g. (97%), m.p. 87–93° was found to be 100% *threo*-α-nitrato-α′-nitrobibenzyl by analysis of its infrared spectrum. One recrystallization from ligroin gave needles, m.p. 96–97°.

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(5) All melting points are uncorrected.

(6) Except on this one occasion when the *threo*-nitronitrate crystallized in a clump and was separated mechanically, the *threo* compound could not be purified by recrystallization. Crystals melting at 85–96°, about 90% pure by infrared analysis, were always obtained.

## Synthesis of DL-β-(5-Cytosinyl)alanine[1]

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*Received February 2, 1959*

With the exception of glycine, natural α-amino acids may be looked upon as β-substituted alanines. In a search for antimetabolites with possible activity against cancer, it seemed desirable to undertake the synthesis of unnatural α-amino acids in which the radical R of the formula, R—$CH_2$—$CH(NH_2)COOH$, would be a pyrimidine, purine, or substitution products thereof. This view was further supported by the fact that a review of the literature revealed no examples of compounds of such comparatively simple structures.

After a number of experiments using conventional methods for preparing various compounds of these types proved abortive in our hands, the procedures described below were tried and led to the successful synthesis of the first compound of this type, DL-β-(4-amino-2-hydroxy-5-pyrimidyl)alanine.

4-Amino-5-hydroxymethyl-2-methylthiopyrimidine (I) was used as a starting point for this series of reactions. The syntheses described by Ulbricht and Price[2] for I and for 4-amino-5-bromomethyl-2-methylthiopyrimidine hydrobromide (II) were modified and improved. These authors reported isolating II as a hygroscopic, noncharacterized solid.[3] In our hands, however, it was obtained as a white crystalline solid exhibiting the chemical and physical properties expected of such a substance. When II was allowed to react with diethyl acetamidomalonate in the presence of alkoxide ion, instead of the expected 2-acetamido-2-(4-amino-2-methylthio-5-pyrimidylmethyl)malonic acid, diethyl ester (III), a cyclic compound, 6-acetamido-5,6,7,8-tetrahydro-2-methylthio-7-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid, ethyl ester (IV), was isolated. Albertson and Archer[4] described a similar occurrence in their synthesis of ornithine

(1) Taken from a portion of the thesis submitted by B. Blank to the Temple University Graduate Council in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June 1958.

(2) T. L. V. Ulbricht and C. C. Price, *J. Org. Chem.*, **21**, 567 (1956).

(3) While this paper was being prepared, the synthesis of this compound (II) was described by T. Okuda and C. C. Price, *J. Org. Chem.*, **23**, 1738 (1958).

(4) N. F. Albertson and S. Archer, *J. Am. Chem. Soc.*, **67**, 2043 (1945).