evaporation yielded 2.64 g. (94%) of a colorless crystalline solid, m.p. 97–100°. Recrystallization from ethanol–water gave shiny rhombs, m.p. 99.5–101.5° (reported[9] 102°).

The infrared absorption spectrum showed the ketone band at 5.96 μ.

3,4,5-Trimethoxyacetophenone (VII).—A suspension of 10 g. of ethyl 3,4,5-trimethoxybenzoylacetate (I) in 100 ml. of 14% sulfuric acid was refluxed for 6 hours, then cooled to yield 7.01 g. (94%) of solid, m.p. 75–77°. Recrystallization from 30% ethanol furnished colorless needles, m.p. 79–80° (reported 73–74°,[8] 77–79°[13]).

Ethyl 3,4,5-Trimethoxybenzoylpyruvate (VIII).—A solution of 5.2 g. of ethyl oxalate in 40 ml. of dry ether was added with stirring to alcohol-free potassium ethoxide (from 0.93 g. of potassium), followed after 10 minutes by a suspension of the ketone VII in 40 ml. of dry ether. A yellow solid separated rapidly. The mixture was refluxed for 1 hour, allowed to stand at room temperature overnight and diluted with cold water. Acidification of the aqueous phase yielded 6.1 g. (81%) of yellow crystals, m.p. 94–96°, which after recrystallization from ethanol–water formed bright yellow needles, m.p. 96–97°.

Anal. Calcd. for $C_{15}H_{18}O_7$: C, 58.06; H, 5.80. Found: C, 58.27; H, 5.64.

The mono-2,4-dinitrophenylhydrazone crystallized in bright yellow needles from ethanol, m.p. 203–205°.

Anal. Calcd. for $C_{21}H_{21}O_{10}N_4$: C, 51.53; H, 4.33. Found: C, 51.65; H, 4.46.

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[CONTRIBUTION FROM THE HARRISON LABORATORY OF CHEMISTRY, THE UNIVERSITY OF PENNSYLVANIA, AND THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Analogs of Windaus' Anhydride. Synthesis of 2,3-Dimethoxy-9-benzoylaminobenzosuber-5-ene-5,6-dicarboxylic Anhydride[1,2]

BY JOHN KOO[3]

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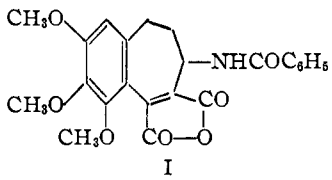
2,3-Dimethoxy-9-benzoylaminobenzosuber-5-ene-5,6-dicarboxylic anhydride (XI), an analog of Windaus' anhydride, and a few related compounds, have been synthesized.

The structure of Windaus' N-benzoylcolchinic anhydride[4] has been established as 2,3,4-trimethoxy-7-benzoylaminobenzosuber-5-ene-5,6-dicarboxylic anhydride (I) by the syntheses of deaminocolchinic anhydride (2,3,4-trimethoxybenzosuber-5-ene-5,6-dicarboxylic anhydride)[5,6] and of *dl*-colchinol methyl ether.[7]



I

In view of the tumor-damaging activity of colchicine[8] and of some of its derivatives,[9] in which I proved to be inactive when tested against Sarcoma 37 in mice, it was thought desirable to develop general methods for the preparation of analogous compounds, in which some of the substituents would be located in different positions. The purpose of this paper is to report on the syntheses of 2,3-dimethoxy-9-benzoylaminobenzosuber-5-ene-5,6-dicarboxylic anhydride (XI) and of the closely related ethyl 2,3-dimethoxy-9-benzoylaminobenzosuber - 5 - ene - 6 - carboxylate (XIII). The anhydride XI differs from Windaus' anhydride I by the position of the benzoylamino group and by the absence of one of the methoxyl groups. The methods for the synthesis of aminobenzosuberenes outlined in this paper might be applicable to the preparation of compounds analogous to colchicine itself.

The present investigation forms an extension of previous work[5,6,10] on the synthesis of benzosuberene derivatives. The required starting material, γ-(3,4-dimethoxybenzoyl)-butyric acid (II) was prepared in improved yield (60 instead of 31%) by carrying out the Friedel–Crafts reaction[10] at −10 to −15°. Treatment of its ethyl ester[10] (III) with hydroxylamine hydrochloride in pyridine furnished the oxime (IV) in 91% yield. Catalytic hydrogenation of this oximino ester did not yield the corresponding amino ester, but gave the lactam V in almost quantitative yield. Alkaline hydrolysis of V and benzoylation of the resulting amino acid VI, which was not isolated, produced the benzoylamino acid VIII. It was found subsequently that this acid could be prepared more readily by catalytic hydrogenation and benzoylation of the oximino acid VII, which in turn was obtained in 88% yield from II with hydroxylamine hydrochloride in pyridine.

Esterification of the benzoylamino acid VIII

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(2) Presented in part before the Organic Division of the American Chemical Society at Boston, Mass., on April 4, 1951.

(3) American Cancer Society Postdoctoral Fellow, 1948–1950. Special Research Fellow of the National Cancer Institute, National Institutes of Health.

(4) A. Windaus, *Ann.*, **439**, 59 (1924).

(5) E. C. Horning, M. G. Horning, J. Koo, M. S. Fish, J. A. Parker, G. N. Walker, R. M. Horowitz and G. E. Ullyot, THIS JOURNAL, **72**, 4840 (1950).

(6) J. Koo, *ibid.*, **75**, 720 (1953).

(7) H. Rapoport, A. R. Williams and M. E. Cisney, *ibid.*, **73**, 1414 (1951).

(8) H. Lettré and H. Fernholz, *J. Physiol. Chem.*, **278**, 175 (1943); B. Goldberg, L. G. Ortega, A. Goldin, G. E. Ullyot and E. B. Schoenbach, *Cancer*, **3**, 124 (1950).

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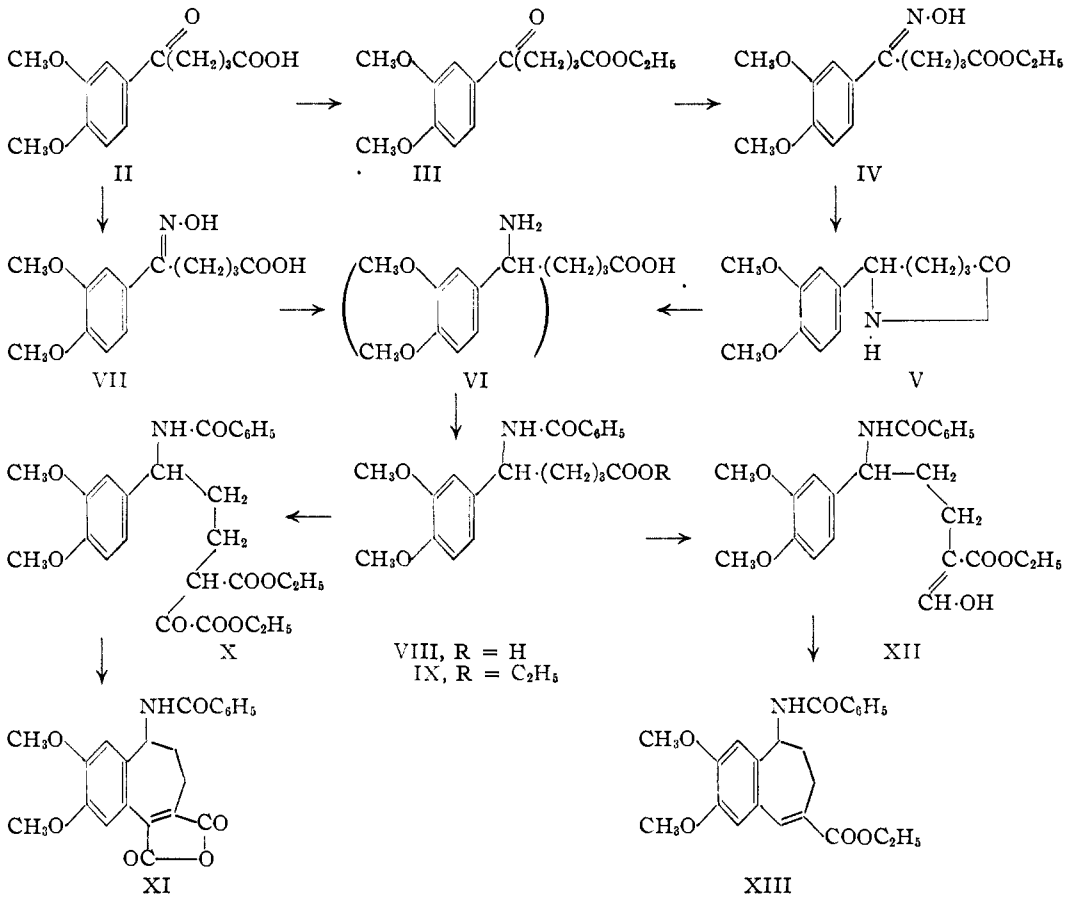
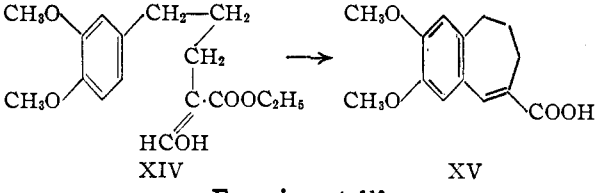


with ethanol and sulfuric acid afforded the ester IX, which, by condensation with ethyl oxalate,[6,10] yielded the keto diester X, from which the anhydride XI was obtained in 35% yield by cyclization with sulfuric–phosphoric acids. Previous work has already indicated that the formation of benzosuberenes by glyoxylate cyclizations[6,10] requires much closer attention to experimental details than the analogous preparations of indenes[11] and dihydronaphthalenes,[12] either because of the sensitivity of the reaction product to the cyclizing agent or because of the occurrence of side-reactions. The presence of the benzoylamino group in the 9-position rendered the cyclization even more difficult. Since extensive decomposition took place rapidly even at 0°, it became necessary to work up the reaction mixture almost immediately and the relatively low yield is therefore not surprising. The anhydride XI had properties similar to those of Windaus' N-benzoylcolchinic anhydride (I) and of the benzosuberenedicarboxylic anhydrides synthesized previously.[5,6,10]

Condensation of the benzoylamino ester IX with ethyl formate was performed by the usual procedure.[11] The formyl ester XII was then cyclized to ethyl 2,3-dimethoxy-9-benzoylaminobenzosuber-5-ene-6-carboxylate (XIII) by treatment with polyphosphoric acid. This reaction had to be carried out at a much lower temperature than in previous instances,[6,11] again because of the greater instability of the reaction product or because of possible side-reactions. By a similar procedure, ethyl δ-(3,4-dimethoxyphenyl)-valerate[10] was condensed with ethyl formate, the formyl derivative XIV cyclized with polyphosphoric acid, and the product saponified to 3,4-dimethoxybenzosuber-5-ene-6-carboxylic acid (XV).



Experimental[13]

Ethyl δ-Oximino-δ-(3,4-dimethoxyphenyl)-valerate (IV).—This compound was prepared from 1.0 g. of ethyl γ-(3,4-dimethoxybenzoyl)-butyrate (III),[10,14] 1.0 g. of hydroxylamine hydrochloride, 5 ml. of pyridine and 5 ml. of ethanol by refluxing for three hours. The solvents were removed under reduced pressure, and the residue triturated with cold water. The crude product was crystallized from 50% ethanol. The yield of material, m.p. 65–69°, was 0.95 g. (91%). Another crystallization from ether–pentane provided colorless needles, m.p. 69.5–71.5°.

Anal. Calcd. for $C_{15}H_{21}O_5N$: C, 61.00; H, 7.17. Found: C, 60.97; H, 7.32.

δ-Amino-δ-(3,4-dimethoxyphenyl)-valeric Acid Lactam (V).—A solution of 5.0 g. of the oximino ester IV in 60 ml. of acetic acid was hydrogenated at room temperature and 40 lb. pressure with 4.0 g. of 5% palladium–carbon catalyst.

(11) E. C. Horning, J. Koo and G. N. Walker, THIS JOURNAL, 73, 5826 (1951).

(12) E. C. Horning and J. Koo, *ibid.*, 73, 5828 (1951).

(13) All melting points are corrected. Infrared measurements were made in chloroform solution with a Perkin-Elmer model 21 spectrometer.

(14) The yield of III was increased to 83% when the ethanol–sulfuric acid solution of II[10] was refluxed for 20 hours.

The theoretical amount of hydrogen was absorbed within one hour. Removal of the catalyst and evaporation of the solvent left 3.9 g. (98%) of crystalline neutral material, m.p. 143–146°. A small sample was recrystallized from 50% ethanol to form colorless needles, m.p. 146–147°.

Anal. Calcd. for $C_{13}H_{17}O_3N$: C, 66.66; H, 6.84. Found: C, 66.58; H, 7.00.

δ-Oximino-δ-(3,4-dimethoxyphenyl)-valeric Acid (VII).—A solution of 15.0 g. of the keto acid II and of 15 g. of hydroxylamine hydrochloride in 50 ml. of pyridine and 50 ml. of absolute ethanol was refluxed for five hours, and the crude product was isolated and purified as in the case of the ester (IV). The yield of colorless crystals was 14.0 g. (88%), m.p. 142–144°.

Anal. Calcd. for $C_{13}H_{17}O_5N$: C, 58.42; H, 6.41. Found: C, 58.52; H, 6.36.

δ-Benzoylamino-δ-(3,4-dimethoxyphenyl)-valeric Acid (VIII). (A).—The clear solution obtained by refluxing 1.5 g. of the lactam V with 15 ml. of 20% sodium hydroxide for three hours deposited, on standing overnight, a crystalline solid, presumably the sodium salt of the amino acid VI. This was brought into solution by the addition of water. Two grams of benzoyl chloride was added dropwise with vigorous shaking and cooling. Water and 5% sodium hydroxide were added alternately until a clear solution resulted. A gummy material separating upon acidification with dilute hydrochloric acid was recrystallized from 50% ethanol to give 1.3 g. (57%) of colorless crystals, m.p. 151–153°. The product was soluble in dilute sodium bicarbonate solution and recovered unchanged after acidification.

Anal. Calcd. for $C_{20}H_{23}O_5N$: C, 67.41; H, 6.17. Found: C, 67.54; H, 6.03.

(B).—A solution of 4.0 g. of the oximino acid VII in 60 ml. of acetic acid was hydrogenated at 60° and 30–40 lb. pressure with 2.0 g. of 5% palladium–carbon catalyst. The theoretical amount of hydrogen was taken up in three hours. After removal of the catalyst, the solution of the amino acid VI was concentrated, made strongly alkaline with 20% sodium hydroxide, and benzoylated with 3.0 g. of benzoyl chloride. The yield of material, m.p. 151–153°, isolated as above, was 2.8 g. (53%). Samples obtained by the two methods showed no depression in mixed melting point.

Ethyl δ-Benzoylamino-δ-(3,4-dimethoxyphenyl)-valerate (IX).—A solution of 5.0 g. of the acid VIII in 30 ml. of dry ethanol and 3 ml. of concd. sulfuric acid was refluxed for 3.5 hours. Most of the alcohol was removed by distillation and the residue treated with ether and water. The ether solution was washed with sodium bicarbonate solution and with water. After drying and removing the ether, there was obtained 4.0 g. (72%) of a neutral, nearly colorless, viscous oil, which could not be distilled without decomposition. In another experiment, an ether solution of the oily product deposited colorless needles, m.p. 123–125° after prolonged standing at room temperature.

Anal. Calcd. for $C_{22}H_{27}O_5N$: C, 68.57;. H, 7.00. Found: C, 68.79; H, 6.57.

Ethyl α-Ethoxalyl-δ-benzoylamino-δ-(3,4-dimethoxyphenyl)-valerate (X).—Following essentially the previously described procedure,[6,10] 4.0 g. of the ester IX in dry ether was condensed with 2.2 g. of ethyl oxalate by means of dry potassium ethoxide prepared from 0.4 g. of potassium. The reaction proceeded rapidly with separation of a brownish-red solid. After standing for eight hours, the mixture was treated as usual, and the keto diester isolated as a viscous red oil, which was used immediately for the cyclization. The yield was 4.0 g. (75%).

2,3-Dimethoxy-9-benzoylaminobenzosuber-5-ene-5,6-dicarboxylic Anhydride (XI).—A solution of 2.0 g. of the keto diester X in 5 ml. of 85% phosphoric acid was added dropwise at 0° to a stirred mixture of 10 ml. of 85% phosphoric acid and 15 ml. of concd. sulfuric acid. The mixture, which became immediately dark brownish-red, was diluted with ice-water after two minutes and the dark yellow precipitate dissolved in ether. The ether solution was washed with water, 5% sodium bicarbonate and again water, then dried over magnesium sulfate. Ether was evaporated in a stream of air at room temperature, and the residue recrystallized from ethyl acetate–ether–pentane to yield 0.7 g. (35%) of bright yellow crystals, m.p. 165–167°. Like Windaus' anhydride[4] and the other anhydrides of this series,[6,10] this compound dissolved in warm aqueous sodium hydroxide to yield a colorless solution; acidification immediately returned the yellow crystalline bicarbonate-insoluble anhydride. The elementary analysis indicated the presence of one molecule of water of crystallization.

Anal. Calcd. for $C_{22}H_{19}O_6N \cdot H_2O$: C, 64.23; H, 5.11. Found: C, 64.32; H, 5.54.

The infrared absorption spectrum exhibited two sharp peaks at 5.48 and 5.67 μ, characteristic of an anhydride grouping, and agreed with the spectra of several other anhydrides of this series.[6,10] The two bands at 6.22 and 6.39μ indicated the monosubstituted amide.[15]

Ethyl α-Formyl-δ-benzoylamino-δ-(3,4-dimethoxyphenyl)-valerate (XII).—A suspension of alcohol-free potassium ethoxide (from 1.56 g. of potassium and 20 ml. of absolute ethanol) in 25 ml. of dry ether was stirred below −20°, while a solution of 7 g. of the benzoylamino ester IX and of 6 g. of ethyl formate in 25 ml. of dry ether was added dropwise. After an additional hour at −20°, the temperature was allowed to come to −15°, and stirring continued for another 3.5 hours. A brownish solid separated. The mixture was allowed to stand at room temperature for three days, then treated with ice-water. The aqueous layer was separated, acidified and extracted with ether. After drying and evaporating the ether, there remained 3.2 g. (43%) of a viscous reddish oil, which gave a red precipitate with 2,4-dinitrophenylhydrazine reagent. It could not be distilled without decomposition and was cyclized immediately.

Ethyl 2,3-Dimethoxy-9-benzoylaminobenzosuber-5-ene-6-carboxylate (XIII).—A mixture of 1 g. of the formyl ester XII and 10 g. of polyphosphoric acid was prepared at 0–5° and stirred at that temperature. After several minutes, it became dark purple. After 10 minutes, it was poured into ice-water, and the light yellow gelatinous material extracted with ether. The ether solution was washed with 5% sodium bicarbonate and with water, and dried over magnesium sulfate. The yellow oil (0.45 g., 45%) remaining after evaporation of the ether was dissolved in benzene–ethyl acetate (5:1), chromatographed on alumina, and eluted with the same solvent mixture. The resulting material separated from ethyl acetate–pentane in fine cream-colored crystals, m.p. 66–68°. The compound did not react with 2,4-dinitrophenylhydrazine reagent and crystallized, like the anhydride XI, with one molecule of water.

Anal. Calcd. for $C_{23}H_{25}O_6N \cdot H_2O$: C, 69.17; H, 6.76. Found: C, 69.36; H, 6.76.

Ethyl α-Formyl-δ-(3,4-dimethoxyphenyl)-valerate (XIV).—This compound was obtained from 4 g. of ethyl δ-(3,4-dimethoxyphenyl)-valerate,[10] 3 g. of ethyl formate and potassium ethoxide from 1.2 g. of potassium by following exactly the procedure employed for the preparation of XII. The yield of yellow oil was 1.9 g. (43%).

2,3-Dimethoxybenzosuber-5-ene-6-carboxylic Acid (XV).—A mixture of 0.9 g. of the crude formyl ester XIV and 10 g. of polyphosphoric acid was prepared at 10° and kept at that temperature for 30 minutes, at which time it had become dark red. It was then poured into ice-water and the gummy material extracted with ether. The residue remaining after evaporation of the ether was refluxed with 15 ml. of 20% aqueous sodium hydroxide for five hours. Acidification of the solution precipitated gummy material. This was redissolved in dilute sodium hydroxide, the solution warmed with Norit and the filtrate again acidified. The yield of pale yellow solid was 0.5 g. (66%). Four recrystallizations from 50% ethanol gave fine colorless crystals, m.p. 158–160°.

Anal. Calcd. for $C_{14}H_{16}O_4$: C, 67.74; H, 6.45. Found: C, 67.83; H, 6.70.

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