[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF G. D. SEARLE AND COMPANY]

THE KNOEVENAGEL CONDENSATION OF 3,4,5-TRIMETHOXYBENZALDEHYDE WITH *p*-METHOXYPHENYLNITROMETHANE

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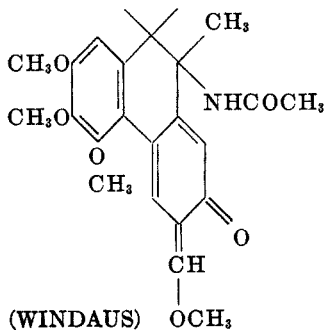
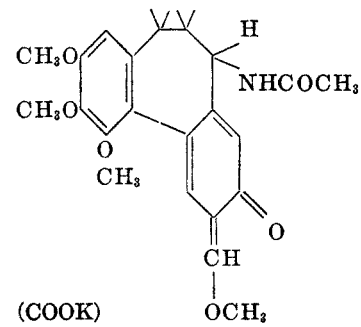
Although recent work on colchicine has not yet resulted in an unequivocal and generally accepted structural formula (1, 2, 3), the structure originally proposed by Windaus (4) definitely seems inadequate. Curiously enough, however, Lettré (5) found that the mitosis-inhibiting properties of certain simple analogs, *e.g.* I, predicated upon the Windaus formula are very similar to those exhibited by colchicine and its degradation products, whereas those analogs, *e.g.* II, based on the Cook formula, do not show this mitotic poisoning property.

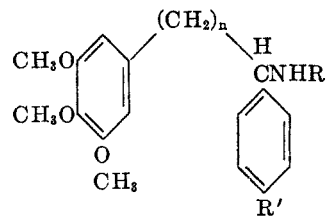
COLCHICINE FORMULAS



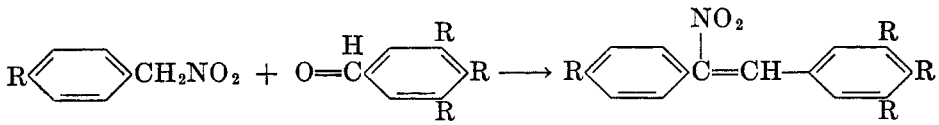
(WINDAUS)



(COOK)

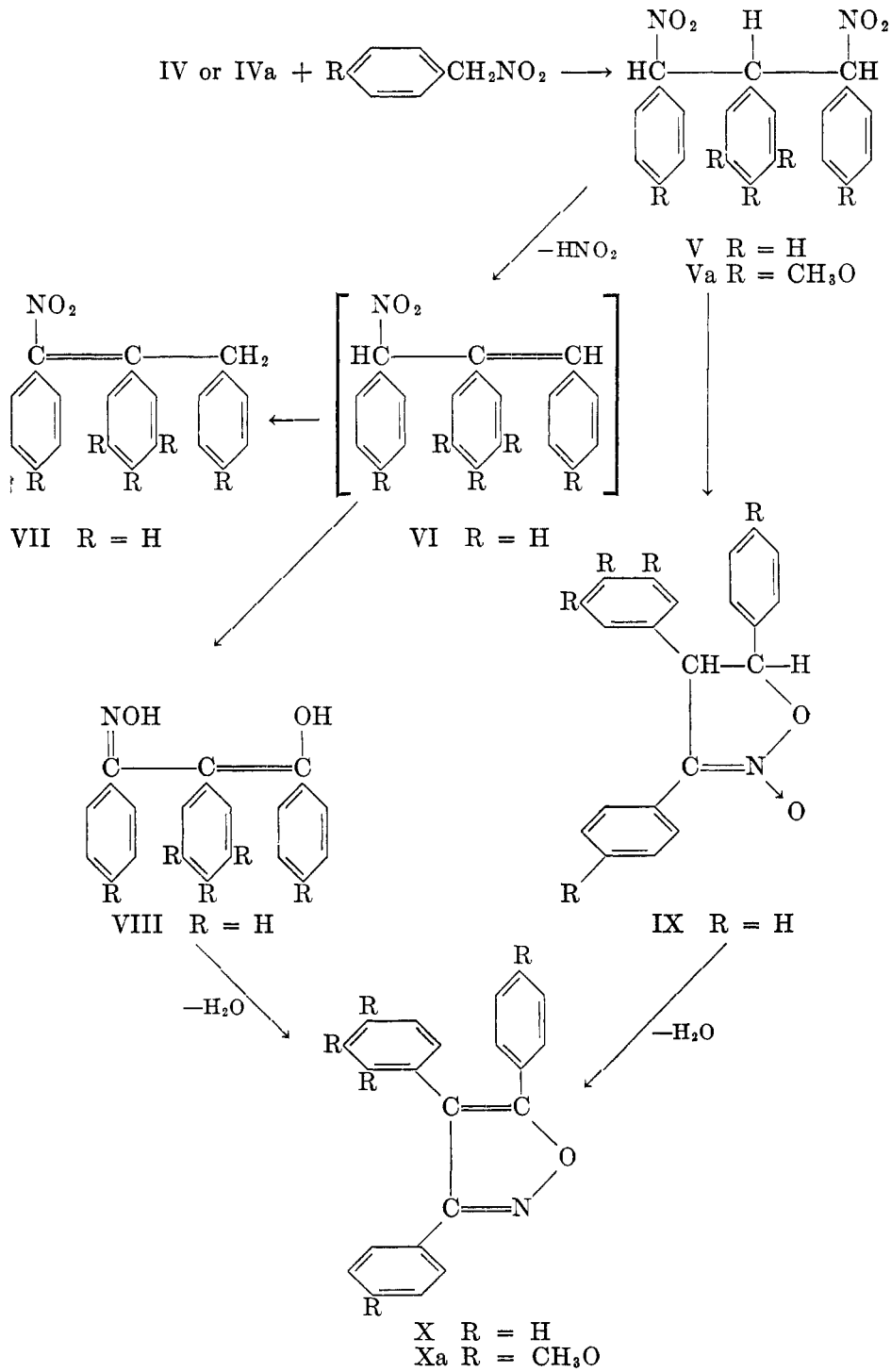


I R = H or Ac; R′ = H; n = 1
II R = H or Ac; R′ = OCH_3; n = 2
III R = H; R′ = OCH_3; n = 1



R = H or CH_3O

IV R = H
IVa R = CH_3O


IV or IVa + R CH2NO2
NO2 H NO2
HC C CH
R R R R R
−HNO2
V R = H
Va R = CH3O
NO2
C C CH2
HC C CH
VII R = H
VI R = H
R
R R
R
CH C H
O
C N
O
R
NOH OH
C C C
R R R
VIII R = H
IX R = H
−H2O
−H2O
R
R
R
C C
O
C N
R
X R = H
Xa R = CH3O

α-(*p*-Anisyl)-β-(3,4,5-trimethoxyphenyl)ethylamine (III) has now been prepared by the catalytic reduction (Pd-C) of 3′,4,4′,5′-tetramethoxy-α-nitrostilbene and tested as a mitosis-inhibitor. The required α-nitrostilbene IVa was prepared by the Knoevenagel condensation of 3,4,5-trimethoxybenzaldehyde with *p*-methoxyphenylnitromethane.

In addition to IVa there were formed in small amounts three other compounds: the dinitropropane[1] (Va), the isoxazole (Xa), and a compound of empirical formula $C_{26}H_{27}NO_7$ (XI). This is in accord with the formation of their unmethylated analogs from phenylnitromethane and benzaldehyde as reported by Heim (6). His compound $C_{21}H_{17}NO_2$, corresponding to our pentamethoxy analog (XI), was regarded by Heim and others (7) as having the nitropropene structure (VII). These workers further thought the isomeric nitropropene (VI) to be an intermediate in the formation of the isoxazole (X) from the nitrostilbene (IV) and phenylnitromethane *via* the path V → VI → VIII → X.

Kohler and Barret (8), however, found as a by-product from this condensation a compound $C_{21}H_{17}NO_2$ differing from that of Heim. This was shown to have the isoxazoline oxide structure (IX). Since IX was obtained also by the condensation of the nitrostilbene (IV) with phenylnitromethane in the presence of sodium methoxide, and was readily transformed into the isoxazole (X) by an excess of sodium methoxide, IX rather than VI was considered an intermediate in the formation of X as a by-product from the Knoevenagel condensation. By using ammonia with a trace of water as the Knoevenagel catalyst, Worrall (9) was able to obtain yet a third entity of formula $C_{21}H_{17}NO_2$, easily transformed into the isoxazole (X), which had all the characteristics expected of the enol-oxime (VIII). This compound had previously been postulated as an intermediate by Heim (6) but had not been isolated by him. Which of these three structures VII, VIII, or IX, if any, shall be regarded as analogous to our compound $C_{26}H_{27}NO_7$ is as yet uncertain. Our compound is not a bright yellow as was Heim's analog, nor is it soluble in aqueous alkali as demanded by Worrall's structure (9). Ruggli and Hegedus (10) have isolated an enol-oxime, which they regard as analogous to Worrall's, from *o*-nitrobenzaldehyde and phenylnitromethane. This compound is soluble in dilute base from which it is reprecipitated unchanged by acid.

It seems, however, that our compound $C_{26}H_{27}NO_7$ was formed *after* the isoxazole Xa and is therefore not a precursor of Xa. It is hoped soon to resume work which will yield the correct structural formula of $C_{26}H_{27}NO_7$ and afford further insight into the reaction mechanism.

Using the single-coverslip method (11) of tissue culture to test for inhibition of multiplication of embryonic chicken heart fibroblasts, it was found[2] that colchicine inhibited growth when present at a concentration of 0.001 mg. per ml. of chicken plasma, whereas 0.3 mg. of III per ml. of plasma was required

[1] Reichert and Hoffman, *Arch. Pharm.*, **274**, 217 (1936), have reported the formation of an analogous dinitropropane from phenylnitromethane and 3,4,5-trimethoxybenzaldehyde.

[2] We are greatly indebted to Dr. James Clampit of these laboratories for this report on the growth-inhibiting properties.

to inhibit growth of these cells. Oddly enough, the α-nitrostilbene (IVa) was more potent than its hydrogenation product III, inhibiting growth at a concentration of 0.03 mg. per ml. of plasma. Compounds Va and Xa were too water-insoluble to permit testing.

EXPERIMENTAL[3]

3,4,5-Trimethoxybenzaldehyde. This material was prepared in 56–79% yield by the Rosenmund reduction (12) of 3,4,5-trimethoxybenzoyl chloride with 5% palladium catalyst supported on barium sulfate (13). No catalyst poison was used but the yields of aldehyde improved as the catalyst was re-used a second and a third time.

p-Methoxyphenylnitromethane. Sodium *p*-methoxyphenyl-*aci*-nitroacetonitrile, obtained in 65% yield from homoanisonitrile (14) and methyl nitrate, was hydrolyzed and decarboxylated according to the general procedure of Meisenheimer and Weibezahn (7) to give a 60% yield of crude *p*-methoxyphenylnitromethane. This undistilled product was not further purified since it usually decomposed violently when small quantities (10 g.) were distilled.

Knoevenagel condensation. Gaseous methylamine was bubbled for one minute into a solution of 58.5 g. (0.3 mole) of trimethoxybenzaldehyde and 50 g. (0.3 mole) of *p*-methoxyphenylnitromethane in 170 ml. of 95% ethanol. After overnight standing at room temperature had yielded no crystals, the mixture was scratched and cooled in the refrigerator for three hours. Thereupon 40 g. of canary-yellow crystals melting at 129–132° were obtained (crop A). Two recrystallizations from absolute ethanol followed by two recrystallizations from a benzene-petroleum ether mixture gave an analytical sample of 3′,4,4′,5′-tetramethoxy-α-nitrostilbene (IVa) melting at 133–134°. [Lit. m.p. 137° (5c)].

Anal. Calc'd for $C_{18}H_{19}NO_6$: CH_3O, 35.95. Found: CH_3O, 35.88, 35.84.

The reaction mixture filtrate, reduced two-thirds in volume by distillation of ethanol *in vacuo*, deposited 6.3 g. of lemon-yellow crystals, m.p. 150–153° (crop B). This, when extracted with hot absolute ethanol, left a residue of 0.5 g. of white crystalline *1,3-di-(p-anisyl)-2-(3′,4′,5′-trimethoxyphenyl)-1,3-dinitropropane* (Va), m.p. 235–237°.

Anal. Calc'd for $C_{26}H_{28}N_2O_9$: C, 60.93; H, 5.51; N, 5.47; CH_3O, 30.28.
Found: C, 61.0; H, 5.52; N, 5.37; CH_3O, 30.22.

The alcohol-soluble portion of crop B was recrystallized from 120 ml. of 80% ethanol to give 4.63 g. of white *3,5-di-(p-anisyl)-4-(3′,4′,5′-trimethoxyphenyl)isoxazole* (Xa), m.p. 156–158°. A thrice-recrystallized analytical sample melted at 158–159°.

Anal. Calc'd for $C_{26}H_{25}NO_6$: C, 69.78; H, 5.63; N, 3.13; CH_3O, 34.68.
Found: C, 69.3; H, 5.67; N, 3.05; CH_3O, 34.62.

A third crop (crop C) of oily, yellow crystals was obtained from the reaction filtrate after it had stood for four months at 5°. By triturating this crystalline sludge with hot ethanol, 0.35 g. of faintly yellow crystals, m.p. 165–167°, was obtained as an insoluble residue. The ethanolic solution from this trituration deposited 2.7 g. of a white solid, m.p. 70–85°, upon dilution with water. Since the melting point of this material was not improved by recrystallization from a variety of solvents, it was not investigated further. A mixture melting point of the above product melting at 165–167° with *isoxazole*, m.p. 158–159°, was 138–150°.

Anal. Calc'd for $C_{26}H_{27}NO_7$: C, 67.08; H, 5.89; N, 3.01; CH_3O, 33.33.
Found: C, 66.51; H, 5.86; N, 2.85; CH_3O, 33.58.

α-(p-Anisyl)-β-(3,4,5-trimethoxyphenyl)ethylamine hydrochloride. Catalytic reduction of the nitrostilbene (IVa) was done according to the directions of McPhee, *et al.* (15). Ten grams of 3′,4,4′,5′-tetramethoxy-α-nitrostilbene, 0.6 g. of palladium chloride, and 4 g. of acid-washed "Darco G-60" charcoal took up the required amount of hydrogen in 2.5 hours of shaking at 50°. After filtration and evaporation of the solvent, the free amine was taken up in 30 ml. of absolute ethanol containing 1.5 g. of anhydrous hydrogen chloride. Warming,

[3] All melting points are uncorrected. The analyses were done in these laboratories by Dr. Robert Dillon and his staff.

adding absolute ether to incipient cloudiness, and cooling gave 5.0 g. of the desired hydrochloride, m.p. 214–217°. A sample twice recrystallized from ethanol-ether melted at 216–218°. A second crop from the original mother liquor weighed 1.6 g., m.p. 190–202°.

Anal. Calc'd for $C_{18}H_{24}ClNO_4$: N, 3.96; Cl, 10.02; CH_3O, 35.08.
Found: N, 3.85; Cl, 9.65; CH_3O, 35.30.

SUMMARY

The Knoevenagel condensation of 3,4,5-trimethoxybenzaldehyde with *p*-methoxyphenylnitromethane has been utilized to prepare 3′,4,4′,5′-tetramethoxy-α-nitrostilbene. Three additional products of this reaction have been isolated and the possible modes of their formation discussed.

Hydrogenation of the above α-nitrostilbene has given α-(*p*-anisyl)-β-(3,4,5-trimethoxyphenyl)ethylamine, an open chain analog of Windaus' colchicine formula.

The growth-inhibitory properties of the above compounds are given.

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