Fragmentation of 3-Oximino-4-chromanone

H. C. WORMSER ˣ **and SU-YA LIEU**

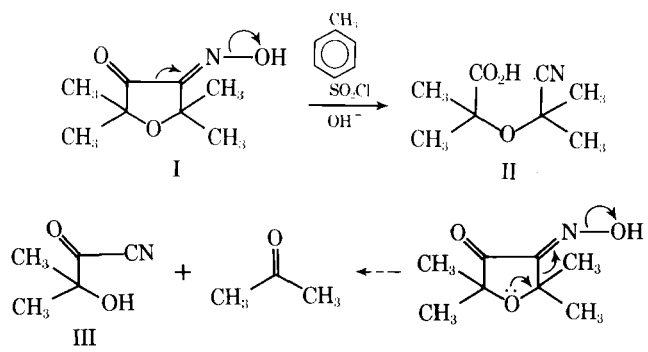
Abstract □ Heating either the methanesulfonate ester of 3-oximino-4-chromanone or 3-oximino-4-chromanone and an alternative acylating agent such as *p*-toluenesulfonyl chloride or acetic anhydride in the presence of aqueous base afforded two major fragments: salicylic acid and 2-carboxyphenoxyacetonitrile. These compounds are derived from two separate cleavage pathways involving the acylated oxime. In one pathway, fragmentation appears to be assisted by the ether ring oxygen; in the other, it is assisted by the α-carbonyl group of the oxime ester.

Keyphrases □ 3-Oximino-4-chromanone—Beckmann fragmentation study after acylating □ Fragmentation—Beckmann rearrangement of 3-oximino-4-chromanone after acylating

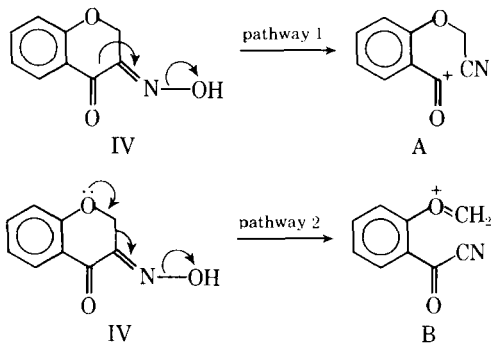
Although the Beckmann rearrangement is principally concerned with the conversion of an oxime to an amide, products other than amides are frequently isolated from the attempted oxime rearrangement. In particular, many oximes take an alternative course of reaction, cleaving into a nitrile, especially when an electron-deficient intermediate can be ejected from the oxime carbon. Consequently, substituents on the α-carbon that stabilize carbonium ions favor this process. Thus, fragmentation reactions are seen with α-trisubstituted ketoximes (1–6), α-disubstituted ketoximes (7–9), α-oximino ketones (10, 11), γ-oximino ketones (12–15), α-oximino acids (16, 17), α-amino oximes (18–23), α-imino oximes (24), α-hydroxy oximes (25–28), β-keto ether oximes (29–32), and β-keto thioether oximes (33, 34). In these cases, rearrangement to amides does not take place. Whereas the fragmentation of an α-oximino ketone is facilitated by the formation of a stable acylium ion, the fragmentation reaction of an α-amino ketoxime or a β-keto ether oxime is assisted by the lone pair electrons of the neighboring nitrogen or oxygen.

DISCUSSION

Oximes containing two of these functionalities (an α-carbonyl group and a β-oxygen) in the same ring system have not been thoroughly studied. Rodina *et al.* (35) reported on the abnormal Beckmann rearrangement of the monoketoxime of 2,2,5,5-tetramethyltetrahydrofuran-3-one-4-oxime (I), which gave the nitrile II when treated with *p*-toluenesulfonyl chloride in base or when simply heated (Scheme I).



Scheme I



Scheme II

In principle, an additional cleavage pathway induced by the ether oxygen is possible. The latter should lead to product III and acetone; this was not reported previously (35).

The objective of this research was to examine the Beckmann fragmentation of a compound capable of a twofold fragmentation process. The model compound chosen was 3-oximino-4-chromanone (IV), which in theory should be capable of cleaving to either of two resonance-stabilized cations, A or B (Scheme II).

Workup of the reaction mixture resulting from the fragmentation of IV could afford two products: 2-carboxyphenoxyacetonitrile and 2-hydroxybenzoyl cyanide, derived from cations A and B, respectively.

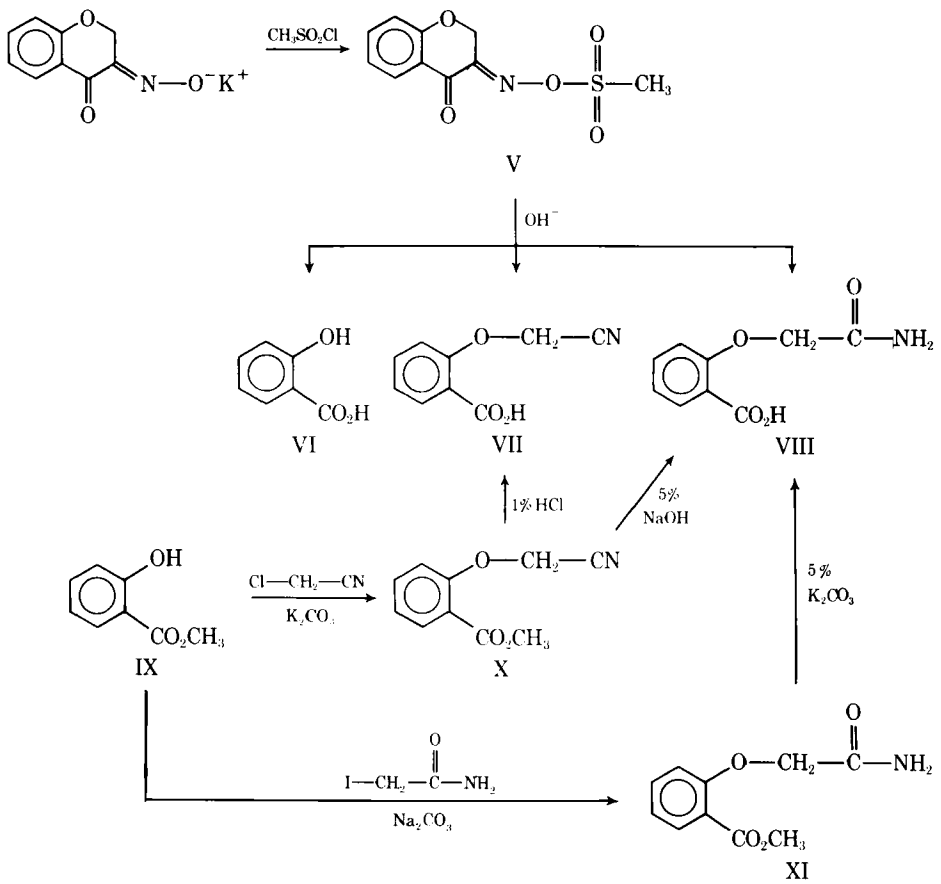
When the methanesulfonate of IV was subjected to one equivalent of sodium hydroxide in dilute solution, three products were obtained, VI, VII, and VIII (Scheme III). Product VI was readily identified as salicylic acid and presumably arises from cation B. It is not clear at this point how this product is actually formed, but several tentative explanations can be put forth:

1. Following hydrolysis to 2-hydroxybenzoyl cyanide, primary fragment B undergoes decarbonylation to 2-hydroxybenzonitrile, which is then hydrolyzed to salicylic acid.
2. Hydrolysis of B gives 2-hydroxybenzoylformic acid, and this compound is subsequently decarbonylated to salicylic acid.
3. 2-Hydroxybenzoylformic acid undergoes decarboxylation rather than decarbonylation, and the resultant aldehyde undergoes a Cannizzaro reaction, affording both salicylic acid and salicyl alcohol.

Needless to say, none of the intermediates mentioned under 1–3 was detected in the crude reaction mixture. Product VII (mp 145–147°) did not give a purple color when treated with ferric chloride solution, but it did dissolve in 5% sodium bicarbonate with the evolution of carbon dioxide. It proved extremely unstable to dilute aqueous base. When dissolved in 5% sodium hydroxide at room temperature and acidified immediately thereafter, a different product (mp 227–229°) was obtained. The latter compound proved identical to VIII and was subsequently identified as 2-carboxyphenoxyacetamide. The IR spectrum of VII showed carboxyl stretching but no absorption in the 4.24–4.55-μm region (nitrile) as anticipated from pathway 1 (Scheme II).

To establish firmly the structures of VII and VIII as postulated from their chemical behavior, spectra, and elemental analyses, these compounds were synthesized independently from methyl salicylate (IX) as shown in Scheme III.

The IR spectra of both ester X and its corresponding acid VII failed to reveal nitrile stretching even in concentrated solutions. Kitson and Griffith (36) observed that in nitriles possessing an α-oxy group, the nitrile band becomes weak or is completely absent. This quenching effect was reported by other investigators (37–40). Substituents such as halogens and methyl groups on the benzene ring do not significantly affect the occurrence or absence of the nitrile band (37–40). However, if the oxygen in these deriva-



Scheme III

tives is replaced by sulfur, selenium, or amino groups or if the oxygen atom is separated from the nitrile group by two or more methylene groups, then the stretching band of nitrile occurs as predicted.

The extreme sensitivity toward base-catalyzed hydrolysis of VII might lend support to some theories postulated previously to explain the formation of VI. More than likely, the *o*-carboxyl group serves as an intramolecular catalyst for the facilitated hydrolytic reaction. Such intramolecularly assisted hydrolyses of esters and amide derivatives have been well documented (41–45).

The Beckmann fragmentation of IV was also examined with additional leaving groups such as *p*-toluenesulfonate and acetate (Scheme IV). Similar results were obtained. In the case where IV was treated with *p*-toluenesulfonyl chloride in 10% base, salicylic acid and 2-carboxyphenoxyacetic acid (XII) were the only products isolated; when acetic anhydride and 5% sodium hydroxide were used, VI, VII, and VIII were identified as the products from the fragmentation reaction.

This degradation pathway is a potentially applicable method for the structural determination of natural products containing the 4-chromanone ring system. Such chromanone-containing substances are found among the flavanones and flavanone glycosides such as pinocembrin, naringin, and hesperidin.

EXPERIMENTAL[1]

3-Oximino-4-chromanone Methanesulfonate (V)—To a stirred suspension of 0.8 g (4.0 mmoles) of the potassium salt of 3-oximino-4-chromanone (IV) (46) in 20 ml of ice-cold anhydrous ether was added dropwise freshly distilled methanesulfonyl chloride (0.5 g, 0.44 mole). The reaction mixture was then stirred for 20 min in an ice bath, during which time the brown suspension gradually turned yellow. The yellow precipitate was filtered, washed with several portions of ice-cold water until free of chloride, and air dried (0.7 g, 75%), mp 121–122°.

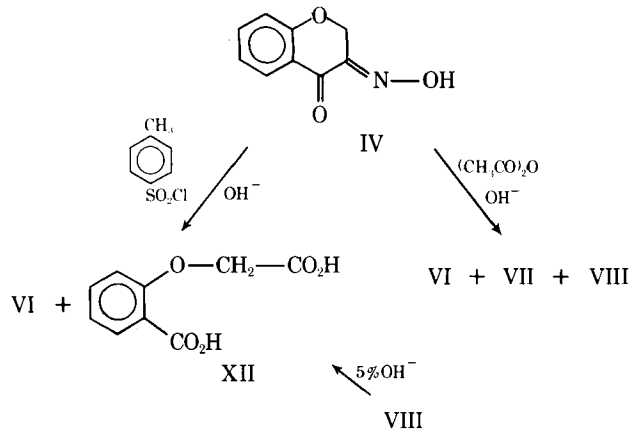
TLC on silica gel G (4% methanol in chloroform) showed a major spot with R_f 0.7; UV: λ_{max} (ethanol) 277 nm; IR: λ_{max} (mineral oil) 5.85, 7.30, and 8.46 μm; NMR (deuterochloroform): δ 3.34 (s, 3H, CH_3), 5.44 (s, 2H, OCH_2), and 7.00–8.13 (m, 4H, aromatic H). Attempts to recrystallize the compound failed due to its instability, so an analytical sample could not be obtained. When V was dissolved in 80% ethanol at 75° for 50 min, TLC on silica gel G (5% methanol in chloroform) showed several new spots in addition to V.

Fragmentation of V—An equimolar quantity (4 ml) of 1% sodium hydroxide was added to 0.5 g (2.0 mmoles) of V, and the mixture was heated on the steam bath until solution was complete. The neutral solution was filtered and cooled. A pale-yellow precipitate formed slowly, and an additional quantity was obtained by adding two to three drops of 10% hydrochloric acid. The precipitate was filtered and dried (0.20 g). TLC on silica gel G [benzene–methanol–acetic acid (45:8:4)] showed one major and two minor spots with R_f 0.6, 0.5, and 0.3, respectively.

Extraction of the aqueous, acidified filtrate with chloroform afforded a crystalline residue (0.03 g), mp 160–161°. TLC of this compound using the same solvent system showed correspondence to the R_f 0.6 spot of the mixture. This compound gave a purple reaction with ferric chloride and was soluble in 5% sodium bicarbonate (effervescence); IR: λ_{max} (chloroform) 3.35 and 5.92 μm; NMR (acetone-d_6): δ 6.80–7.98 (m, 4H, aromatic protons) and 11.03 (s, 2H, OH and COOH). This substance was characterized as salicylic acid (VI) on the basis of superimposable spectral data and undepressed mixed melting point with an authentic sample. Preparative TLC on silica gel $PF_{254+366}$ of the initially obtained solid precipitate [benzene–methanol–acetic acid (45:8:4)] afforded the three substances as crystalline residues: VI (0.094 g, mp 159–161°), VII (0.031 g, mp 142–145°), and VIII (0.024 g, mp 220–223°).

Compound VII was recrystallized from chloroform, yielding colorless plates, mp 145–147°; IR: λ_{max} (mineral oil) 5.90 μm; NMR

[1] Melting points were taken on a Fisher-Johns melting-point stage and a Thomas-Hoover melting-point apparatus and are uncorrected. UV absorption spectra were determined in 95% ethanol on a Beckman (model DK2A) recording spectrophotometer. IR absorption spectra were recorded on Beckman (models 8 and 33) recording spectrophotometers. NMR spectra were determined on a Varian EM 360 spectrometer. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. TLC was carried out with silica gel G, silica gel $HF_{254+366}$, and silica gel $PF_{254+366}$.



Scheme IV

(dimethyl sulfoxide-d_6): δ 5.26 (s, 2H, OCH_2) and 7.00–8.00 (m, 4H, aromatic H).

Anal.—Calc. for $C_9H_7NO_3$: C, 61.02; H, 3.98; N, 7.91. Found: C, 60.90; H, 3.99; N, 7.86.

On the basis of the data obtained, VII was characterized as 2-carboxyphenoxyacetonitrile.

Compound VIII (corresponding to R_f 0.3) was recrystallized from methanol, mp 227–229°; IR: λ_{max} (mineral oil) 2.93, 3.09, 5.90, and 6.00 μm; UV: λ_{max} (ethanol) 231 and 289 nm; NMR (deuterium oxide-sodium deuteroxide): δ 4.65 (s, 2H, OCH_2) and 6.80–7.55 (m, 4H, aromatic H).

Anal.—Calc. for $C_9H_9NO_4$: C, 55.37; H, 4.65; N, 7.18. Found: C, 55.40; H, 4.73; N, 7.20.

On the basis of the data obtained, VIII was identified as 2-carboxyphenoxyacetamide.

2-Carbomethoxyphenoxyacetonitrile (X)—A mixture of methyl salicylate (IX) (15.2 g, 0.10 mole), chloroacetonitrile (8.0 g, 0.11 mole), and anhydrous potassium carbonate (14.0 g, 0.10 mole) in 200 ml of acetone was refluxed overnight. The solvent was removed under reduced pressure, and the remaining slurry was partitioned between chloroform and water. The organic phase was washed with several portions of 2% sodium hydroxide and dried over anhydrous sodium sulfate. Following filtration and evaporation to dryness, a light-yellow oil was obtained (12.5 g, 65%). Upon standing, the oil crystallized, mp 52–53°. Recrystallization from petroleum ether gave colorless needles, mp 53–54°; IR: λ_{max} (chloroform) 5.81 μm; NMR (deuterochloroform): δ 3.92 (s, 3H, CH_3), 4.88 (s, 2H, OCH_2), and 6.98–8.03 (m, 4H, aromatic H).

Anal.—Calc. for $C_{10}H_9NO_3$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.86; H, 4.78; N, 7.29.

2-Carboxyphenoxyacetonitrile (VII)—A suspension of 0.20 g (1.0 mmole) of X in 10 ml of 1% hydrochloric acid was heated to reflux for 2 hr. The oily reaction mixture became clear during this time and was then allowed to cool. The crystalline product that had formed was filtered and dried (0.17 g, 97%), mp 141–145°. Recrystallization from chloroform gave shiny, colorless plates, mp 145–147°. The mixed melting point with VII obtained from the fragmentation of V was undepressed, and their IR and NMR spectra were superimposable.

2-Carbomethoxyphenoxyacetamide (XI)—A mixture of IX (7.6 g, 0.05 mole), iodoacetamide (9.3 g, 0.05 mole), and anhydrous sodium carbonate (6.9 g, 0.05 mole) in 125 ml of acetone was refluxed for 6 hr. The solvent was removed under reduced pressure, and the crystalline residue was washed with several portions of water and dried (5.4 g, 52%), mp 140–145°. Recrystallization from acetone gave shiny needles, mp 147–148°; IR: λ_{max} (mineral oil) 2.93, 3.10, 5.80, and 5.90 μm; NMR (dimethyl sulfoxide-d_6): δ 3.87 (s, 3H, OCH_3), 4.60 (s, 2H, OCH_2), and 6.83–8.16 (m, 4H, aromatic H).

Anal.—Calc. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.33; H, 5.28; N, 6.65.

2-Carboxyphenoxyacetamide (VIII)—*Method A*—A suspension of 0.20 g (1.0 mmole) of XI in 10 ml of 5% potassium carbonate was stirred for 12 hr at room temperature. The reaction mixture was cooled, filtered, and acidified with concentrated hydrochloric acid to congo red. The precipitate was filtered and dried (0.084 g, 45%), mp 210–220°. Two recrystallizations from methanol gave fine colorless needles, mp 227–229°. The mixed melting point with VIII obtained from the fragmentation of V was undepressed, and their IR and NMR spectra were superimposable.

Method B—2-Carboxyphenoxyacetonitrile (0.20 g, 1.0 mmole) was dissolved in 10 ml of 5% sodium hydroxide, and as soon as all of the nitrile had gone into solution, the mixture was acidified with 10% hydrochloric acid to congo red. The white precipitate was filtered and dried (0.13 g, 59%), mp 220–222°. Recrystallization from methanol afforded colorless needles, mp 226–228°. The mixed melting point with the product obtained by Method A was undepressed.

Reaction of 3-Oximino-4-chromanone (IV)—*With p-Toluenesulfonyl Chloride in Presence of Aqueous Base*—A solution of IV (0.20 g, 1.0 mmole) in 10 ml of 10% aqueous sodium hydroxide was treated with 0.8 g (4.0 mmoles) of *p*-toluenesulfonyl chloride. The mixture was heated on the steam bath for 20 min, cooled, and filtered. The clear filtrate was acidified with 10% hydrochloric acid, and the turbid mixture was extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and, following filtration, concentrated to dryness. TLC of the solid residue on silica gel G [benzene–methanol–acetic acid (45:8:4)] showed two spots of roughly equal intensity with R_f 0.2 and 0.6.

Sublimation of the solid mixture at atmospheric pressure (75–80°) gave a shiny, colorless sublimate, mp 159–160°. TLC analysis and ferric chloride color reaction indicated that this product was salicylic acid. The residue remaining after the sublimation contained primarily the compound with the lower R_f (0.2). Recrystallization from methanol–chloroform–petroleum ether gave a crystalline product, mp 191–193°; IR: λ_{max} (mineral oil) 3.16, 5.75, and 5.90 μm; NMR (dimethyl sulfoxide-d_6): δ 4.84 (s, 2H, OCH_2) and 7.00–7.90 (m, 4H, aromatic H). This product was identified as 2-carboxyphenoxyacetic acid (XII) [lit. (47) mp 186° and (48) mp 190–193°].

With Acetic Anhydride in Presence of Aqueous Base—To a solution of IV (0.40 g, 2.0 mmoles) in 20 ml of 5% aqueous sodium hydroxide was added 4.3 g (0.042 mole) of acetic anhydride dropwise over 10 min. After the exothermal reaction ceased, the mixture was acidified with 10% hydrochloric acid and extracted with several portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. TLC of the solid residue on silica gel G [benzene–methanol–acetic acid (45:8:4)] showed two major and one minor spots with R_f 0.6, 0.5, and 0.3, respectively.

The compound with R_f 0.6, giving a violet color with ferric chloride solution, was identified as salicylic acid by comparison of the TLC mobility of an authentic sample. The compound with R_f 0.5 was identical with VII, while the minor component with R_f 0.3 was found to be identical with VIII by comparison of thin-layer chromatograms.

2-Carboxyphenoxyacetic Acid (XII)—A suspension of VIII (1.0 g, 5 mmoles) in 10 ml of 5% sodium hydroxide was refluxed overnight. As soon as the reaction began to reflux, a strong evolution of ammonia was detected at the mouth of the reflux condenser. The pink solution was cooled and acidified with concentrated hydrochloric acid, and the crystalline precipitate was filtered and dried (0.10 g, mp 191–193°). Recrystallization from methanol–chloroform–petroleum ether gave shiny, colorless crystals, mp 192–193°. A mixed melting point with the product obtained from the fragmentation of IV (*p*-toluenesulfonyl chloride and base) was undepressed, and their IR and NMR spectra were superimposable.

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Analysis of Tetracycline in Pharmaceutical Preparations by Improved High-Performance Liquid Chromatographic Method

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Abstract □ The analysis of tetracycline in pharmaceutical preparations by an improved high-performance liquid chromatographic (HPLC) method is described. The improved method uses a 30-cm long stainless steel column packed with octadecylsilane bonded on 10-μm silica gel, with a linear gradient from 10 to 60% acetonitrile in pH 2.5, 0.02 *M* phosphate buffer in 11 min at a flow rate of 1.0 ml/min (68 atm). The resolution functions obtained between 4-epitetracycline and tetracycline and between 4-epianhydrotetracycline and anhydrotetracycline were improved 150 and 250%, respectively. The analysis of a tetracycline sample takes approximately 16 min; the original method required more than 25 min. The relative standard deviation for the analysis of tetracycline powder was 0.66%, and the recovery of 4-epianhydrotetracycline added in tetracycline was linear over the 0.3–100% range. Recovery of tetracycline from products was better than 99.6% at label concentration. The drug content of products as calculated from the HPLC data agreed well with those of the microbiological assay methods.

Keyphrases □ Tetracycline—analysis, high-performance liquid chromatography, pharmaceutical preparations □ High-performance liquid chromatography—analysis, tetracycline in pharmaceutical preparations □ Antibiotics—tetracycline, analysis, high-performance liquid chromatography, pharmaceutical preparations

On March 4, 1974, the Food and Drug Administration (FDA) established the limit for 4-epianhydrotetracycline in tetracycline pharmaceutical dosage forms marketed in the United States (1). However, the limit is higher than that outlined in the European Pharmacopoeia (2).

Prior to the development of high-performance liquid chromatographic (HPLC) assay methods for tetracycline (3–6), the methods used for the detection and quantitation of tetracycline impurities (7, 8) were very tedious, and none has been accepted by FDA as an alternative method for potency determination. There is also some indication that degradation compounds may be forming on-column during