was isolated in 65% yield in the form of colorless prisms, m.p. 167–170° (bath preheated to 160°). Found: C, 64.14; H, 5.06. Calc. for $C_{16}H_{14}ClN_3O$: C, 64.11; H, 4.71.

The Isomerization of II to the Nitrone I. Method A.—A spectrophotometric study of a solution of the oxaziridine II in an excess of 0.1 *N* hydrochloric acid (room temperature) indicated that after 26 hr. a 99% conversion into the nitrone I had occurred.

Method B.—A 1.5% isopropyl alcohol solution of the oxaziridine II, which was refluxed for 50 min., was shown spectrophotometrically to contain 90% of the nitrone I. The mixture was refluxed for an additional 3 hr., concentrated under reduced pressure, cooled, and allowed to crystallize. Ninety per cent of the nitrone I was recovered; m.p. and mixed m.p. 236–236.5°.

Acknowledgment.—We are indebted to Dr. V. Toome and Dr. A. Motchane for most of the spectrophotometric determinations, and to Dr. Al Steyermark and his staff for the microanalyses.

Nonclassical Antimetabolites. X.[1,2] A Facile Synthesis of 4-Quinazolone-2-carboxylic Acid and the Structure of Bogert's Ammonium Salt

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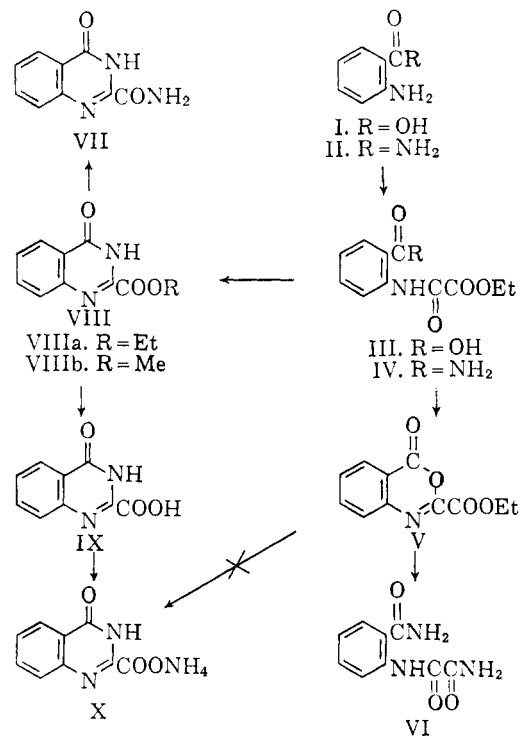
Recently, we presented[3,4] strong experimental evidence to support the concept[5] of a new class of irreversible inhibitors that operate by exo-alkylation. A properly designed compound, such as 4-(iodoacetamido)salicyclic acid, can complex reversibly with an enzyme such as glutamic dehydrogenase, then become irreversibly bound within the complex adjacent to the active site. A number of factors can influence the rate at which the reversibly complexed inhibitor can become covalently bound to the enzyme such as (1) the ability of the reversibly bound inhibitor to bridge to a nucleophilic site on the enzyme,[6,7] (2) the nucleophilic character of the enzymic group,[2] (3) the reactivity of the group on the inhibitor forming the bond, and (4) the K_i of the reversibly bound inhibitor.

The latter phenomenon would be important in chemotherapy, since the lower the K_i, the less intracellular concentration of inhibitor will be needed to inactivate a given enzyme. Compounds bearing 1,2- or 1,3-oxo (or hydroxyl) and carboxyl groups usually give good reversible inhibition of lactic and glutamic dehydrogenases; in fact, 1-hydroxy-2-naphthoic acid,[8] coumarin-3-carboxylic acid,[9] and 2-hydroxycinchoninic acid,[9] can reversibly bind to these enzymes much more effectively than salicylic acid and in some cases even better than the substrate. Since 4-quinazolone-2-carboxylic (IX) has the proper relationship of oxo and carboxyl functions, this compound has been resynthesized for evaluation as an inhibitor; however, it was found to bind less effectively than salicylic acid (I_{50} about 20)[8] since IX had $I_{50} = 31$ for glutamic dehydrogenase and $I_{50} = 33$ for lactic dehydrogenase. Thus, IX was not suitable for conversion to a potent irreversible inhibitor.



Among the routes available[10,11] for synthesis of 4-quinazolone-2-carboxylic acid, the route (I→III→V→X→IX) described by Bogert and Gortner[10] seemed attractive in view of past use[12] of anthranils such as V for synthesis of a variety of 4-quinazolones. Preparation of the anthranil, V, and reaction with ethanolic ammonia according to the described procedure[10] gave a compound iden-

(1) This work was generously supported by Grant CY-5867 of the National Cancer Institute, U. S. Public Health Service.

(2) For paper IX of this series see B. R. Baker and R. P. Patel, *Biochem. Biophys. Res. Comm.*, **9**, 199 (1962).

(3) B. R. Baker, W. W. Lee, E. Tong, and L. O. Ross, *J. Am. Chem. Soc.*, **83**, 3713 (1961); paper III of this series.

(4) B. R. Baker, W. W. Lee, and E. Tong, *J. Theor. Biol.*, **3**, 458 (1962); paper VI of this series.

(5) B. R. Baker, *Cancer Chemotherapy Repts.*, No. **4**, 1 (1959), published by the National Cancer Institute; paper I of this series.

(6) B. R. Baker, *J. Med. Pharm. Chem.*, **5**, 654 (1962); paper VII of this series.

(7) B. R. Baker, *Biochem. Pharm.*, in press; paper VIII of this series.

(8) B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, *J. Med. Pharm. Chem.*, **2**, 633 (1960); paper II of this series.

(9) B. R. Baker, W. W. Lee, E. Tong, L. O. Ross, and A. P. Martinez, *J. Theor. Biol.*, **3**, 446 (1962); paper V of this series.

(10) M. T. Bogert and R. A. Gortner, *J. Am. Chem. Soc.*, **32**, 123 (1910).

(11) A. Reissert and F. Grube, *Ber.*, **42**, 3713 (1909).

(12) B. R. Baker, M. V. Querry, A. F. Kadish, and J. H. Williams, *J. Org. Chem.*, **17**, 35 (1952), and following papers.

tical in properties to that of the earlier preparation, which Bogert had assigned the structure of ammonium 4-quinazolone-2-carboxylate (X). Although this compound, on reanalysis, corresponded to the empirical formula of X, the relative insolubility in water and a sharp NH band at 3450 cm.$^{-1}$ made this structure unattractive. Our skepticism became greater when we were unable to hydrolyze the supposed X to the corresponding free acid (IX)[11] with concentrated hydrochloric acid as previously described.[10]

Two other possibilities for Bogert's "ammonium salt" were the anthranilamide derivative VI, which is isomeric to X, and 4-quinazolone-2-carboxamide (VII), which would be isomeric to X if it formed a relatively stable hydrate; both VI and VII would be expected to have the primary amide NH band at about 3500 cm.$^{-1}$, but X would not have this observed band. In order to differentiate these two structures, the "ammonium salt" was heated with boiling methanolic sulfuric acid which could be expected to convert VII to methyl 4-quinazolone-2-carboxylate (VIIIb)[13] and convert VI to methyl anthranilate. Since small yields of methyl anthranilate could be isolated by this treatment, structure VI was favored for the "ammonium salt"; however, methyl anthranilate could also possibly, but less likely, arise by ring cleavage of VII. That the "ammonium salt" had structure VI was established as follows:

Fusion of anthranilamide (II) with ethyl oxalate at 180° did not lead to the intermediate ethoxalyl derivative (IV), but resulted in spontaneous ring closure to the ultimately desired 2-carbethoxy-4-quinazolone (VIIIa), m.p. 179–180°. The latter compound had an ultraviolet spectrum identical with VIIIb,[13] and was further identified by combustion analyses; in addition, basic hydrolysis gave in 92% yield the desired 4-quinazolone-2-carboxylic acid (IX). This acid (IX) is relatively unstable in boiling alcohol, being decarboxylated to 4-quinazolone; it was best purified by acid precipitation from its solution in aqueous sodium bicarbonate. This simple two step synthesis for IX is preferred over those previously described.[10,11] The acid (IX) was readily soluble in ammonium hydroxide and the solution did not deposit insoluble "ammonium salt (X)."

Reaction of 2-carbethoxy-4-quinazolone (VIIIa) with ethanolic ammonia gave the corresponding carboxamide (VII) which gave analytical figures for the anhydrous compound; the analyses and infrared spectrophotometric data clearly indicated that it was not identical with the "ammonium salt." Thus, the only possible remaining structure for Bogert's "ammonium salt" was the isomeric anthranilamide derivative, VI. That this "ammonium salt" did have structure VI was clearly shown by comparative ultraviolet and infrared spectra.

All of the quinazolone derivatives (VII, VIII, and IX) had inflections in the ultraviolet at 318 mμ and no peak in the 255–275-mμ region. In contrast, N-acetylanthranilamide and N-ethoxalylanthranilic acid (III) each had a peak in the 255–275-mμ region and no peak or inflection higher than 305 mμ; the "ammonium salt" had the ultraviolet spectrum therefore expected for VI with a peak at 270 mμ and no peak or inflection beyond 305 mμ.

In the infrared, the anthranilic derivatives showed strong bands at 1580 and 1510–1520 cm.^{1} which were absent in the quinazolones (VII–IX), but present in the "ammonium salt." These bands are attributed to C=C of benzoyl derivatives. In addition, compounds III, VII, N-acetylanthranilamide and the "ammonium salt" clearly showed a sharp NH band at 3300–3450 cm.$^{-1}$, a band that was not present in VIIIa or IX, clearly showing that the "ammonium salt" was an amide. Thus, Bogert's "ammonium salt" was both an anthranilic derivative and an amide and could therefore only have structure VI.

Experimental[14]

N-Oxamylanthranilamide (VI) (Bogert's "Ammonium Salt").—To a stirred solution of 95% alcohol saturated with ammonia and cooled in an ice bath was added 2.0 g. (0.01 mole) of V[10] in one portion. Solution rapidly took place and in a few minutes a white solid separated. After being stirred in the ice bath for an additional 15 min. the mixture was heated to boiling, then cooled, filtered, and the product washed with ethanol; yield, 2.0 g. (99%), m.p. 242° with evolution of ammonia. Recrystallization from water gave white silky needles, m.p. 241° dec.; ν_{max}^{KBr} 3440, 3240 (NH); 1670, 1650 (amide C=O); 1600, 1570 (amide II); 1520 cm.$^{-1}$ (C=C); $\lambda_{max}^{Me.\ Cell.}$ 230 (ε 16,800, inflection), 270 (ε 10,100), 302 mμ (ε 7400).

Anal. Calcd. for $C_9H_9N_3O_3$: C, 52.1; H, 4.36; N, 20.2. Found: C, 52.2; H, 4.60; N, 19.9.

Bogert and Gortner[10] recorded a m.p. of 229° dec. with evolution of ammonia and recrystallized the compound from water. When treated with 12 *N* hydrochloric acid at room temperature for several hours, the compound was recovered unchanged and none of IX could be isolated, in contrast to the results of Bogert.[10] When refluxed with methanolic sulfuric acid for 20 hours, methyl anthranilate was obtained.

Ethyl 4-Quinazolone-2-carboxylate (VIIIa).—A mixture of 6.80 g. (0.05 mole) of anthranilamide and 14.6 g. (0.1 mole) of ethyl oxalate was heated in an oil bath at 170–180° for 6 hr.; the melt solidified on cooling. The solid was dissolved in 300 ml. of hot absolute ethanol; the solution was filtered hot to remove a little of the insoluble bis-substituted oxamide (0.12 g.), then cooled. The product was collected on a filter and washed with cold ethanol; yield, 6.2 g. (57%) of pale yellow crystals, m.p. 179–180°; no additional product could be recovered from the filtrate. Recrystallization from absolute ethanol gave white crystals of unchanged m.p.; ν_{max}^{KBr} 1730 (ester C=O); 1670 (amide C=O); 1600 (amide II and C=N); 1170 cm.$^{-1}$ (ester C—O—C); λ_{max}^{alc} 229 (ε 19,800); 297 (ε 11,200); 318 (ε 2800, inflection).

(13) During the course of our work, a new ring-expansion synthesis of methyl 4-quinazolone-2-carboxylate (VIIIb) was described by W. E. Noland and D. A. Jones, *J. Org. Chem.*, **27**, 342 (1962).

(14) Melting points were determined in capillary tubes in a Mel-Temp block and are uncorrected.

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.5; H, 4.59; N, 12.8. Found: C, 60.3; H, 4.45; N, 12.9.

The spectral data agree with that reported for the corresponding methyl ester (VIIIb).[13] If the above fusion is above 190°, the yields are poor. Knape[15] has reported that the intermediate ethoxalyl anthranilamide (IV) is obtained by fusing the above components at 170°.

4-Quinazolone-2-carboxylic Acid (IX).—A mixture of 500 mg. (2.3 mmoles) of the corresponding ester (VIIIa) and 10 ml. of 5% aqueous sodium hydroxide was stirred at room temperature for 2 hr. The filtered solution was cooled in an ice bath and acidified with 3 *N* hydrochloric acid to about pH 3. The product was collected on a filter, washed with water, and dried over phosphorus pentoxide in a vacuum dessicator at room temperature; yield, 400 mg. (92%), m.p. 212–215° dec. For analysis the compound was dissolved in 20 ml. of 2% aqueous sodium bicarbonate, the solution was filtered, then acidified with 3 *N* hydrochloric acid with ice-cooling; the product was collected and washed with filtered water. This reprecipitation was repeated once more to give white crystals, m.p. 215–216° dec.; ν_{max}^{KBr} 2100–3100 (broad acidic OH); 1670 (amide C=O); 1640 (carboxyl C=O); 1600 (amide II and C=N); 1980, 1550 cm.$^{-1}$ (weak zwitterion); $\lambda_{max}^{Me.\ Cell.}$ 229 (ϵ 20,500); 285 (ϵ 9,950); 305 (ϵ 7,350, inflection); 318 mμ (ϵ 4720).

Anal. Calcd. for $C_9H_6N_2O_3$: C, 56.9; H, 3.16; N, 14.7. Found: C, 56.7; H, 3.34; N, 14.4.

Recrystallization from ethanol lead to partial decarboxylation to 4-quinazolone as shown by ultraviolet and combustion analyses. Knape[15] has recorded a m.p. of 201–202°, prepared by a different route. This compound readily dissolved in concentrated ammonium hydroxide and did not precipitate on insoluble ammonium salt; an attempt to isolate the soluble ammonium salt by evaporation *in vacuo* and recrystallization from aqueous methanol appeared to lead partially to decarboxylation to 4-quinazolone.

4-Quinazolone-2-carboxamide (VII).—To a stirred and ice-cooled saturated solution of ammonia in 95% alcohol was added 500 mg. (2.2 mmoles) of the ester (VIIIa). Solution took place immediately and within 10 min. the product began to separate. After 30 min., the product was collected on a filter; yield, 100 mg. After standing overnight, the solution deposited an additional 200 mg. (total, 80%) of product as white leaflets, m.p. 230°, that was identical with the first crop. This second crop was analyzed and had ν_{max}^{KBr} 3400, 3270 (NH); 1670, 1650 (amide C=O); 1600 cm.$^{-1}$ (amide II and C=N); $\lambda_{max}^{Me.\ Cell.}$ 229 (ϵ 20,400); 297 (ϵ 9,600); 318 mμ (ϵ 5200, inflection).

Anal. Calcd. for $C_9H_7N_3O_2$: C, 57.2; H, 3.70; N, 22.2. Found: C, 57.1; H, 3.84; N, 22.2.

N-Acetylanthranilamide.—Strenuous conditions for acetylation of anthranilamide could be expected to give 2-methyl-4-quinazolone. Although no attempt was made to ascertain optimum conditions, the ring closure could be avoided as follows: To a solution of 1.00 g. (7.5 mmoles) of anthranilamide in 10 ml. of 50% acetic acid was added 0.62 ml. (15 mmoles) of acetic anhydride. Within 15 min., the product began to separate. Crystallization was complete after standing overnight; yield, 250 mg. (21%), m.p. 180°. Recrystallization from methanol gave white crystals of unchanged m.p.; ν_{max}^{KBr} 3400, 3200 (NH); 1670 (amide C=O); 1630, 1600 (amide II); 1580, 1520 cm.$^{-1}$ (C=C); $\lambda_{max}^{Me.\ Cell.}$ 219 (ϵ 25,200); 255 (ϵ 15,600); 302 mμ (ϵ 5,100).

Anal. Calcd. for $C_9H_{10}N_2O_2$: C, 60.6; H, 5.61; N, 15.7. Found: C, 60.5; H, 5.46; N, 15.6.

Ethoxalylanthranilic Acid (III).—Fusion of 20 g. (0.146 mole) of anthranilic acid with 44 g. (0.30 mole) of ethyl oxalate at 140–150° for 1 hr. as described by Bogert[10] gave 20 g. (58%) of product, m.p. 180°; ν_{max}^{KBr} 3300 (NH); 1730 (ester C=O); 1720 (carboxyl C=O); 1670 (amide C=O); 1600 (amide II); 1580, 1520 cm.$^{-1}$ (C=C); λ_{max}^{alc} 235 (ϵ 13,500); 275 (ϵ 8,800); 287 (ϵ 8,300, inflection); 305 mμ (ϵ 8,500).

Bogert and Gortner[10] recorded a m.p. of 184°. Acetone is much more efficient than boiling water for separation of the product from the insoluble by-product of disubstituted oxamide.

(15) E. Knape, *J. prakt. Chem.* [2], **43**, 209 (1891).

Reaction of *meso*- and *dl*-Dimethylsuccinic Acids with Acetyl Chloride[1]

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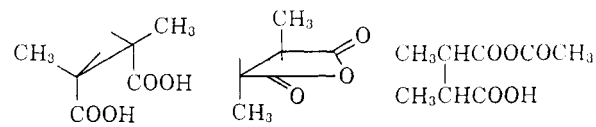
The observation was made by Bone and Sprankling[3] that *dl*-dimethylsuccinic acid (*dl*-I) undergoes ring closure to the *trans* anhydride (*trans*-II) when dissolved in cold acetyl chloride solution under reaction conditions which fail to convert the *meso* isomer (*meso*-I) to the *cis* anhydride (*cis*-II), but give recovered *meso* acid. These results suggested the possible explanation that the *cis* effect of the two methyl groups is responsible for the reluctance of the *meso* isomer to cyclize.[4] Further investigation of this reaction, however, has shown that such is not the case. In fact, the over-all rate of the reaction is not the rate of ring-closure at all but appears to depend on the rate of formation of an intermediate, probably the mixed anhydride III.



dl-I *trans*-II III

The difference in the rates of reaction in acetyl chloride medium apparently is due primarily to the difference in the rates of solution of *dl*- and *meso*-I.

Evidence for this point of view was obtained from two sources. First, when dilatometric rates were measured in dioxane solution, the order of reactivities was reversed, the *meso* isomer being somewhat faster than the *dl*. Second, when succinic acid was treated with ketene in dioxane solution, it was converted to the cyclic anhydride in a reaction with an estimated one-half time of not more than two minutes, very much faster than when acetyl chloride is employed. The formation of the initial linear

(1) Taken from the Ph.D. thesis submitted by Richard Charles Thamm to the University of Illinois, 1957, and available from Univ. Microfilms, Ann Arbor, Mich., as Publication No. 23395.

(2) Rohm & Haas Fellow, 1955–1956. We are indebted to E. I. du Pont de Nemours and Company for a Grant-in-Aid which supported a part of this work.

(3) W. A. Bone and C. H. G. Sprankling, *J. Chem. Soc.*, **75**, 856 (1899); *ibid.*, **77**, 654, 1300 (1900).

(4) D. Y. Curtin, *Record Chem. Progr.* (Kresge-Hooker Sci. Lib.) **15**, 122 (1954).