acid was added and the solution was partially concentrated under vacuum and diluted with 75 ml. of water. After aging overnight in the ice-box the product was filtered, washed with water and dried *in vacuo* to give 0.55 g. of crude 17α-hydroxypregnenolone, m.p. 200–215°. A sample recrystallized from ethanol showed a m.p. 205–210° and did not depress the melting point of an authentic sample of 17a-hydroxypregnenolone.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.85; H, 9.70. Found: C, 75.41; H, 9.66.

5α-Chloropregnan-3β,17α-diol-20-one (VI).—A slurry of 2.5 g. (0.0057 mole) of 3β-acetoxy-5α-chloro-17,20-epoxypregnane-20-ol formate in 125 ml. of methanol was treated at room temperature with 12.5 ml. of concentrated hydrochloric acid. A greenish-blue color developed and within 1 hour solution was practically complete. After standing overnight the reaction mixture was cooled 1 hour in the refrigerator. The heavy precipitate of product was filtered, washed with methanol and dried *in vacuo;* yield 1 g., m.p. 200–205°. Slurrying with boiling ethyl acetate raised the melting point to 205–210°, $[\alpha]^{24}D$ +1 (pyridine); infrared spectrum 2.82 μ, 2.91 μ (OH), 5.90 μ (carbonyl).

Anal. Calcd. for $C_{21}H_{33}O_3Cl$: C, 68.36; H, 9.02; Cl, 9.61. Found: C, 68.33; H, 9.03; Cl, 9.51.

17α-Hydroxyprogesterone (VII).—Chromic anhydride–pyridine complex was formed by adding 1.3 g. (0.013 mole) of chromic anhydride in small portions with stirring to 13 ml. of pyridine while keeping the temperature below 25°. To this stirred slurry was added 1.3 g. (0.0035 mole) of 5α-chloropregnane-3β,17α-diol-20-one in 26 ml. of pyridine. After stirring overnight the brown solution was poured into 500 ml. of dilute sodium hydroxide solution. The precipitated product was aged 1 hour, filtered and washed with water. The product was dissolved in 25 ml. of pyridine and 50 ml. of methanol, filtered to remove insoluble material and treated with 10 ml. of 2.5 *N* sodium hydroxide solution at room temperature for 1 hour. The reaction mixture was then diluted with 100 ml. of water and refrigerated for 1 hour. The filtered product was washed with water and dried *in vacuo;* yield 0.6 g. (51%) of 17α-hydroxyprogesterone, m.p. 200–212°; ultraviolet spectrum λ_{max}^{MeOH} 2420 Å., $E_{1cm}^{1\%}$ 488. A sample recrystallized from ethanol melted at 213–218°, $[\alpha]^{CHCl_3}D$ +91°. Identity of the sample with authentic material was established by mixed melting point and identity of the infrared absorption curves. The authentic sample had the following characteristics, m.p. 212–215°, $[\alpha]^{CHCl_3}D$ +93.8°, λ_{max}^{MeOH} 2420 Å., $E_{1cm}^{1\%}$ 498, phase solubility 99.6%, (lit.[13] m.p. 212–215°, $[\alpha]^{CHCl_3}D$ +102°).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.59; H, 9.17.

(13) J. J. Pfiffner and H. B. North, *J. Biol. Chem.*, **132**, 459 (1940); **139**, 855 (1941).

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE U. S. NAVAL MEDICAL RESEARCH INSTITUTE AND THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

The Acetylcholinesterase Surface. VII. Interference with Surface Binding as Reflected by Enzymatic Response to Turicine, Betonicine and Related Heterocycles[1]

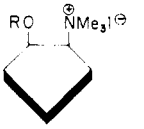
BY S. L. FRIESS, A. A. PATCHETT AND B. WITKOP

RECEIVED AUGUST 6, 1956

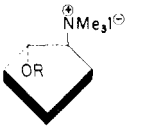
The response of the enzyme acetylcholinesterase to inhibition by the betaine amino alcohols D- and L-turicine (III, IV) and L-betonicine (V) is remarkably sensitive to the relative spatial orientations of the $-COO^-$ and –OH groups. When *trans* to one another as in V inhibition of the low order of strength shown by choline results, while in the *cis* derivatives III and IV no inhibition is observed even at concentrations two orders of magnitude higher. The anilide of V essentially repeats this picture, indicating that the effect of the 2-substituent on the pyrrolidine ring is steric and not electrostatic in character. This difference is discussed in terms of two-point attachment to the catalytic surface *via* the –OH and $-{}^+N\equiv$ functions, and steric interference with this binding when the 2-substituent is necessarily oriented toward the protein surface as compared with no hindrance to binding when the group is directed away from the surface. Additional information on the nature of the binding in these heterocycles comes from comparison of the relative inhibitory strengths of III, IV and V with those of the betaine of hydroxypipecolic acid (VIII), stachydrine (IX) and the methiodide of N-methylpyrrolidine (X). Measurements with compound IX in particular indicate that removal of the –OH function leads to a drop in inhibitory power as compared to betonicine. The acetates of betonicine and turicine appear to be accepted as substrates for the enzyme, but the enzymatic hydrolytic rates are not appreciably higher than those for the "uncatalyzed" water reaction of the betaine esters.

Introduction

Previous studies[2] have centered around a subtle variation in response of the enzyme acetylcholinesterase (AChE) to relatively weak reversible inhibitors of the choline type in the cyclohexane and cyclopentane series, as well as to the corresponding acetylated substrates. For example, the enzyme derived from electric eel tissue is inhibited somewhat more strongly by Ia than by IIa, and of the pair of substrates Ib and IIb the former evokes higher catalytic rates from the enzyme. These results were interpreted in terms of two-point binding to the enzymatic surface *via* the polar quaternized nitrogen function at one site and the –OH or –OAc function at the second site[3] of the catalytic unit.



Ia, R = H
Ib, R = Ac



IIa, R = H
IIb, R = Ac

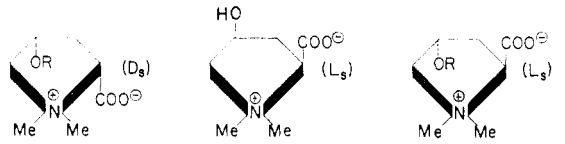
On this assumption, it was of considerable interest to study the effect of steric influences in properly constituted inhibitors or substrates that might tend to impede this two-point adsorption process and lead to lower indices of enzymatic activity. One series of compounds ideally suited

(1) The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department.

(2) H. D. Baldridge, W. J. McCarville and S. L. Friess, THIS JOURNAL, **77**, 739 (1955); H. D. Baldridge and S. L. Friess, *ibid.*, **78**, 2482 (1956).

(3) For a summary of work leading to the postulation of a bifunctional catalytic unit on the enzymatic surface see D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.*, **12**, 259 (1951).

for this purpose was found in some derivatives of naturally occurring heterocycles related to hydroxyproline, namely, the D- and L-forms of the betaine turicine (III, IV) where the –OH and $-COO^{\ominus}$ functions are *cis* to one another and L-betonicine (V) where these functions are in the *trans* relationship. With OH and $Me_2N^+<$ functions to serve as the potential points for attraction to the surface of the enzyme, the *cis*-derivatives III and IV should experience considerable difficulty in bringing the charged nitrogen function close enough to the surface for maximum electrostatic interaction with the anionic site, because of the bulky carboxylate



III, R = H
IIIa, (R = Ac)

IV

V, R = H
Va, (R = Ac)

ion projecting toward that surface. On the other hand, V should have no such difficulty since on adsorption of OH and $Me_2N^+<$ functions the carboxylate group would project away from the surface.

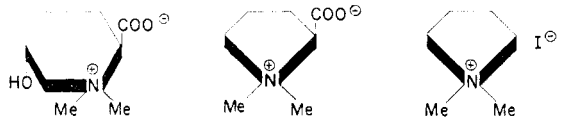
In a further sequence of derivatives to assess the steric contribution toward surface interaction of the 2-substituent as contrasted to its possible electrostatic influence in the charged form $-COO^{\ominus}$, the anilide VII was prepared to provide the steric bulk at position 2 but no negative charge and tested for its inhibitory activity. The anilide VI of turicine was not formed under conditions that led smoothly to the formation of VII. As a result of steric hindrance methylation stopped at the stage of N-methylallohydroxy-D-proline anilide which was isolated as the hydriodide VIa. Allohydroxyproline itself is methylated to the betaine under identical conditions without any difficulty. Also in-

VI, R = CH_3
VIa, R = H

VII

cluded in the inhibitors tested to provide further information on points relating to ring size and the nature of polar functions involved in the enzymatic binding were the betaine[4] VIII of natural 5-hydroxy-L-pipecolic acid,[5] stachydrine (IX) and the quaternary iodide of pyrrolidine X.

Finally, some ancillary data pertaining to the stereochemical acceptance of structures III and V by the surface were sought in the action of the corresponding acetates of turicine (IIIa) and betonicine (Va) as substrates for the enzyme.



VIII IX X

(4) A. A. Patchett and B. Witkop, THIS JOURNAL, **79**, 185 (1957).

(5) B. Witkop and C. M. Foltz, *ibid.*, **79**, 197 (1957).

Results

Inhibitors.—In studies at 25.14° and *p*H 7.4 a very remarkable degree of specificity was shown by AChE in its sensitivity toward inhibition of catalyzed acetyl choline hydrolysis by compounds III–V. Betonicine (V), with its OH and $COO^{\ominus}$ groups *trans* to one another, operates as an effective inhibitor of the enzyme in the relatively high concentration range $1–10 \times 10^{-4}$ *M* with an enzyme-inhibitor dissociation constant[6] K_I of $8.5 \pm 0.4 \times 10^{-5}$, showing this substance to be about five times as effective as choline.[2] In contrast, the *cis* derivative D-turicine (III) shows no signs of inhibiting AChE activity up to concentration levels as high as 6×10^{-2} *M*. In a similar display of this specificity pattern the anilide VII of L-betonicine (VII), where the negative charge of the carboxylate group has been eliminated and the steric bulk of the group at position 2 increased, inhibits at a concentration of 1.1×10^{-3} *M* to the extent of 40%. The near equivalence in inhibitory strengths between the anilide of betonicine and betonicine itself is indicated by the further comparison that at the same 1.1×10^{-3} *M* level where the anilide shows 40% inhibition, betonicine is only slightly better at 48%.

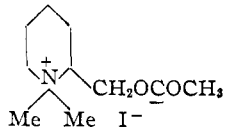
The factor of absolute configuration at position 2, the Ds- and Ls-series, can well be eliminated as a contributing cause to this great difference in power between *cis* and *trans* isomers, since L-turicine (IV) at 1.3×10^{-3} *M* concentration inhibits somewhat less than 1%, or beneath the level of significance under these conditions, reflecting the same lack of activity found for the D-isomer III.

This pronounced difference in ability to inhibit AChE as a function of the *cis–trans* relation of groups at the 2- and 4-positions of the five-membered ring has a reasonable interpretation in terms of a previous assumption[2] that interaction of quaternary amino alcohols with the AChE surface is two-sited in character, with the $—N^+\equiv$ and —OH functions being bound at the anionic and esteratic sites, respectively. In the present examples betonicine and its anilide could be absorbed readily with the quaternary nitrogen atom approaching the surface sufficiently close for electrostatic binding, since either the bulky $-COO^{\ominus}$ or $-CONHC_6H_5$ group at position 2 *trans* to the OH at ring position 4 would be directed away from the surface. However, in turicine orientation of the ring to simultaneously bind OH and $—N^+\equiv$ would necessarily bring the bulky *cis*-2-substituent into proximity to the surface, interfering with the approach of the ring nitrogen to electrostatic interaction distance for binding as an inhibitor.

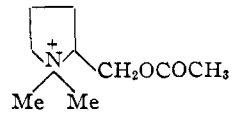
This interpretation of the relative behavior of III and V ascribes the majority of the inhibitory interaction with protein to the polar functions both in and attached to the ring, with a lesser contribution from the five-membered ring itself. Further information bearing on this point is seen in the comparative behavior of the six-membered analog of betonicine, the betaine of 5-hydroxy-L-pipecolic acid (VIII). This compound shows inhibition of

(6) Calculated from the v/v$_I$ *vs.* [I] competitive inhibitor plot of P. W. Wilson as in reference 2.

the same order of magnitude as that displayed by betonicine, with a K_I value (25.14°, *p*H 7.4) of 18 ± 0.5 × 10^{-5} reflecting an inhibitory strength down by a factor of 2 from that of V. This is about the same as the range of effects observed previously[2] on comparison of six *vs.* five-membered rings in the series corresponding to I and II where all substituents are exocyclic and activities could be fairly well correlated with spatial distribution of the substituents. In this same connection, a much smaller dependence of effect of ring size on esterase activity for a closely related pair of heterocycles was noted by Glick,[7] who found that for horse serum esterase the relative hydrolytic activities toward the substrates XIII and XIV were very nearly the same.



XIII



XIV

Further points bearing on the nature of the functions required for binding to the catalytic surface are found in the inhibitory actions of stachydrine (IX) and the simple quaternary derivative of pyrrolidine (X). In comparison with betonicine as standard inhibitor at equivalent concentration levels (1.3–1.5 × 10^{-3}) it was observed that: (1) stachydrine which lacks the OH function of betonicine is just 0.26 as active an inhibitor of AChE; and (2) the activity of the quaternary salt X, lacking both the OH and $COO^{\ominus}$ functions, is similarly lowered to 0.40 of that of betonicine (V). These data indicate that elimination of the OH function does impair the ability of even a relatively weak inhibitor like betonicine to be adsorbed at the catalytic surface of AChE and support the bifunctional view that OH binding complements $-N^+ \equiv$ binding in these choline-like inhibitors. Also, in the derivatives IX and X where the OH is not present and the question of two-point attachment as dictated by the stereochemistry of the ring no longer applies, the monofunctional inhibition through the single quaternary center appears to be adversely affected by the presence of the α-carboxylate residue, since X is a slightly better inhibitor than IX. This could well reflect some degree of repulsion between the anionic site of the enzyme used in binding the quaternary center and the negative end of the dipolar betaine IX.

Substrates.—Since the spatial distribution of the OH and $COO^{\ominus}$ functions in the betaines III, IV and V appear to play such an important role in the binding of these inhibitors to the AChE surface, it was of interest to observe whether this specificity pattern carried over to turicine acetate IIIa and betonicine acetate (Va) as possible substrates of the enzyme. Working at 25.14° and *p*H 7.0—7.1, we found some unexpected hydrolytic properties of these esters. A set of representative hydrolytic results is shown in Fig. 1.

It is seen that the relative rates of the spontaneous, non-enzymatically catalyzed hydrolysis of IIIa and Va are quite high under these conditions,

(7) D. Glick, *J. Biol. Chem.*, **130**, 527 (1939).

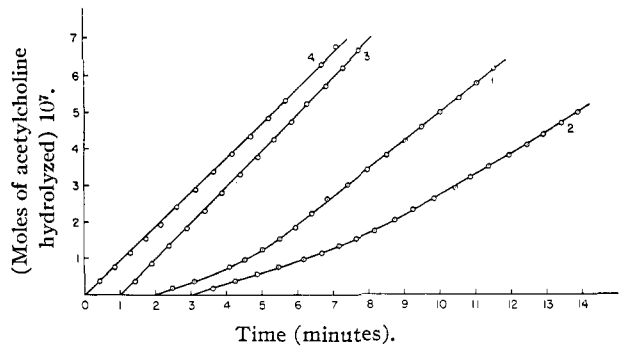


Fig. 1.—(1) Non-enzymatic hydrolysis of acetylturicine and (2) acetylbetonicine at 25.14°, *p*H 7.1, and initial concentrations of 1.97 × 10^{-3} *M*; (3) enzymatic hydrolysis of acetylturicine and (4) of acetylbetonicine at *p*H 7.0 and 1.94 × 10^{-3} *M* concentration.

at initial ester levels of 1.97 × 10^{-3} *M* in these runs. These rates are of the order of 25–50 times those measured for spontaneous acetylcholine hydrolysis[8,9] and increase somewhat with the duration of reaction. In comparison, the AChE-catalyzed hydrolysis rates at this substrate level are higher (curves 3 and 4) for IIIa and Va than the spontaneous rates, but not markedly so; for turicine acetate (curves 1 and 3) the spontaneous rate starts out at 37% of the total AChE rate and rises to about 76% of that value in about 4 minutes of reaction, while for betonicine acetate the corresponding figures are 33% at the start and 59% later. Consequently, little quantitative significance can be attached to the increment in velocity afforded by the enzyme's presence since this is smaller than the uncatalyzed contribution itself, but, qualitatively and to the small extent that it operates, it would appear that the enzyme shows little selection between these substrates.

Experimental[10]

Kinetic Experiments.—Determinations to assay the effects of these quaternary inhibitors on the rates of the AChE-catalyzed hydrolysis of acetylcholine were carried out by the constant-*p*H titration technique of the previous studies.[2,8] Acetylcholine chloride solutions were prepared freshly before use from doubly recrystallized material. Enzyme solutions were aged in dilution from a stock preparation[11] and held at 4° prior to use. The standard enzymatic activity in these runs assayed at 1.09 × 10^4 μ moles acetylcholine hydrolyzed/hr./mg. of protein at a substrate level of 3.36 × 10^{-3} *M*. Kinetic runs were made at 25.14 ± 0.03° in a final reaction volume of 6.40 ml.

For each of those inhibitors to which K_I values were assigned, a series of inhibitor concentration runs over a ten to twenty-fold range was employed in the Wilson plot of v/v_I *vs.*

(8) S. L. Friess and W. J. McCarville, THIS JOURNAL, **76**, 1363 (1954).

(9) It has been observed that typical betaine esters and betaine amides (with the derivatives formed at the $-COO^{\ominus}$ group) are saponified at rates up to 100 times greater than those for similar uncharged compounds; see H. G. Walker and H. S. Owens, *ibid.*, **74**, 2547 (1952), and R. P. Bell and G. M. Waind, *J. Chem. Soc.*, 1979 (1950). In the acetates IIIa and Va, however, it is not the betaine carboxylate group which is esterified but rather an OH function twice removed from the quaternary center.

(10) All melting points are corrected. The analytical and optical rotation data were obtained by courtesy of Dr. W. C. Alford and his Associates, Microanalytical Laboratories, National Institutes of Health, Bethesda, Md.

(11) Prepared from electric eel tissue by the method of M. A. Rothenberg and D. Nachmansohn, *J. Biol. Chem.*, **168**, 223 (1947).

[I], with the slope and the least squares fit yielding the K_I value and its estimated uncertainty. In the case of substrates IIIa and Va, the relative rates noted in the discussion were taken from direct comparison of slopes of corresponding segments of the hydrolysis rate curves; the enzymatic rates for these substrates at the concentration level of 1.97×10^{-3} *M* amounted to 85 to 90% of those for the natural substrate acetylcholine at its concentration optimum of 3.3×10^{-3} *M*. These substrates were added to the reaction media in the form of their purified hydrochlorides, and the ensuing neutralizations by the dilute buffer led to *p*H levels of 7.0–7.1 at which the ester hydrolyses were studied.

Preparation of Inhibitors and Substrates. Betaines of Hydroxyproline.—The known betaines of hydroxy-L-proline and allohydroxy-D-proline, betonicine and turicine, respectively, and acetylbetonicine hydrochloride were prepared by action of silver oxide and methyl iodide on the amino acids as described previously.[4] The betaine of allohydroxy-L-proline L-turicine, was similarly prepared and characterized by m.p. 252–254°, $[\alpha]^{20}D$ −39.0° (*c* 1.0 in H_2O); turicine,[4] m.p. 259–260°, $[\alpha]^{25}D$ +37.8°. The methiodide of N-methylpyrrolidine has been described by Willstätter.[12]

N-Carbobenzyloxyhydroxy-L-proline Anilide.—The mixed anhydride procedure with ethyl chlorocarbonate was used to couple aniline with N-carbobenzyloxyhydroxy-L-proline according to the method of Boissonas.[13] The anilide was obtained in 70% yield, m.p. 149–150°, on crystallization of the crude coupling product from ethanol, ethyl acetate and petroleum ether (b.p. 65°). A sample recrystallized a second time had m.p. 150°, $[\alpha]^{20}D$ −49.8° (*c* 1.0 in methanol).

Anal. Calcd. for $C_{19}H_{20}N_2O_4$: C, 67.04; H, 5.92; N, 8.23. Found: C, 67.17; H, 5.88; N, 8.20.

Hydroxy-L-proline Anilide.—The carbobenzyloxy group was hydrogenolyzed from N-carbobenzyloxyhydroxy-L-proline anilide with 10% palladium-on-charcoal in glacial acetic acid. After evaporation of the solvent *in vacuo*, hydroxy-L-proline anilide was crystallized from ethanol, ethyl acetate and petroleum ether. The analytical sample had m.p. 150–151°, $[\alpha]^{25}D$ −32.8° (*c* 1.0 in methanol).

Anal. Calcd. for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 63.89; H, 6.80; N, 13.67.

When the hydrogenolysis was conducted in methanol in the presence of a molar quantity of HCl, hydroxy-L-proline anilide hydrochloride was obtained, m.p. 205–206°.

Anal. Calcd. for $C_{11}H_{14}N_2O_2 \cdot HCl$: C, 54.43; H, 6.23; N, 11.54. Found: C, 54.15; H, 6.38; N, 11.24.

N-Methyl-hydroxy-L-proline Anilide Methiodide.—Hydroxy-L-proline anilide (0.540 g.) in 10 cc. of methanol was treated at room temperature with silver oxide (0.610 g.) and 1 cc. of methyl iodide. A precipitate of silver iodide rapidly formed and the solution warmed noticeably. After 1 hr. an additional 1 cc. of methyl iodide was added and at the end of 2 hr. the solution was diluted with methanol, filtered and acidified with HI. The residue after evaporation of the solvent *in vacuo* was crystallized from absolute ethanol and ether. In this manner N-methylhydroxy-L-proline anilide methiodide (0.415 g., 44%) was obtained as platelets, m.p. 186–187°, $[\alpha]^{20}D$ +45.2° (*c* 1.0 in water).

Anal. Calcd. for $C_{12}H_{19}N_2O_2I$: C, 43.10; H, 5.29; N, 7.74. Found: C, 43.31; H, 5.18; N, 7.71.

Allohydroxy-D-proline Anilide Hydrochloride.—Aniline and N-carbobenzyloxyallohydroxy-D-proline were coupled by the mixed anhydride procedure as described above for N-carbobenzyloxyhydroxy-L-proline anilide. N-Carbobenzyloxyallohydroxy-D-proline anilide resisted crystallization, but it was convertible to the crystalline allohydroxy-D-proline anilide hydrochloride by hydrogenolysis of the carbobenzyloxy group with 10% palladium-on-charcoal in methanol with a molar equivalent of HCl. Allohydroxy-D-proline anilide hydrochloride crystallized from ethanol and ether as fine needles, m.p. 256–258°, $[\alpha]^{20}D$ +10.2° (*c* 1.0 in water).

Anal. Calcd. for $C_{11}H_{15}N_2O_2Cl$: C, 54.43; H, 6.23; N, 11.54. Found: C, 54.63; H, 6.34; N, 11.43.

N-Methylallohydroxy-D-proline Anilide Hydriodide.—Allohydroxy-D-proline anilide hydrochloride (0.500 g.) was dissolved in 20 cc. of methanol and treated with silver oxide (0.238 g.). After 15 minutes, an additional amount of silver oxide (0.476 g.) and 1 cc. of methyl iodide were added. A further 0.5 cc. of methyl iodide was added after 30 minutes, and at the end of 1 hr., the solution was diluted with methanol, filtered and acidified with HI. Removal of solvent left an oil which on trituration with ethanol and ether yielded 0.228 g. (31%) of crude hydriodide. Several recrystallizations from absolute ethanol afforded an analytical sample of N-methylallohydroxy-D-proline aniline hydriodide m.p. 220–222°. The same compound was obtained when free allohydroxy-D-proline anilide was subjected to the same procedure.

Anal. Calcd. for $C_{12}H_{16}N_2O_2 \cdot HI$: C, 41.39; H, 4.92; N, 8.05; I, 36.45. Found: C, 41.66; H, 4.85; N, 7.93; I, 36.90.

Acetylturicine Hydrochloride.—The conditions used for methylation were those described for acetylbetonicine.[4] The crude crystalline methylation product was converted to its hydrochloride at 0° in ethanol and ethyl acetate with gaseous HCl. Even cautious recrystallization of the product from ethanol and ethyl acetate gave an impure acetylturicine hydrochloride, m.p. 185–187° and $[\alpha]D$ +10.7° (*c* 1.0 in water), whose analysis agreed with that of a mixture containing 75% acetylturicine hydrochloride and 25% turicine hydrochloride.

Anal. Calcd. for $C_9H_{16}NO_4Cl$ (75%) and $C_7H_{14}NO_3Cl$ (25%): C, 44.88; H, 6.90; N, 6.19; CH_3CO, 13.6. Found: C, 44.78; H, 6.95; N, 6.22; CH_3CO, 13.9.

Acknowledgment.—We are indebted to Dr. H. S. Polin for his generous aid in procurement of electric eel tissue extracts.

BETHESDA 14, MD.

(12) R. Willstätter and Heubner, *Ber.*, **40**, 3873 (1907).

(13) Boissonas, *Helv. Chim. Acta*, **34**, 874 (1951).

[CONTRIBUTION FROM THE SCIENTIFIC AND RESEARCH DEPT., SOUTH AFRICAN IRON AND STEEL INDUSTRIAL CORPORATION, LTD.]

The Structures of Isoxazoline Compounds: A Spectral Study

BY G. W. PEROLD, A. P. STEYN AND F. V. K. VON REICHE

RECEIVED JUNE 12, 1956

Ultraviolet absorption characteristics were determined for a series of five isoxazolines, obtained by addition of benzonitrile oxide to various olefins. Correlation with the absorption of model compounds shows that the addition compounds are 2-isoxazolines. The use of infrared absorption in connection with this problem is evaluated.

Isoxazoline compounds are obtained by the interaction of hydroxylamine with β-halogenated ketones[1] as well as with α,β-unsaturated ketones[2] under alkaline conditions. Quilico and his school have in recent years shown[3] that they also are

(1) H. Rupe and F. Schneider, *Ber.*, **28**, 965 (1895).

(2) A. H. Blatt, THIS JOURNAL, **53**, 1133 (1931).

(3) A. Quilico, G. Stango d'Alcontres and P. Grünanager, *Nature*, **166**, 226 (1950); *Gazz. chim. ital.*, **80**, 479 (1950), and further papers from this school.