lagen are formed only as the *trans* diastereoisomers.[49,50]

Nonenzymatic displacements of a hydrogen at saturated carbon atoms with retention of configuration are relatively rare. One such case is the oxidation of *cis*-decalin to *cis*-9-hydroxydecalin, and of *trans*-decalin to *trans*-9-hydroxydecalin by the action of ozone.[51] We may deal here with an internal electrophilic displacement (SEi or "SEi") in which the ozone dipole acts simultaneously as acceptor of the proton and donor of the oxygen function in a concerted front-side displacement. The comparable autoxidation, with the diradicaloid oxygen as participant, should proceed by abstraction of a hydrogen radical from *trans*-decalin only to yield *trans*-9-decalyl hydroperoxide.[52] The stereoselectivity and retention of configuration is remarkable for a radical reaction.

The resonance spectrum of *trans*-4-hydroxy-L-proline in D_2O has been interpreted in favor of a nonplanar regular pentagon with hydroxyl in a *quasi-axial* position.[53] Nonenzymatic hydroxylation of proline[54] by the Udenfriend–Wieland system[55,56] is nonspecific and introduces hydroxyl to yield all four possible hydroxyprolines.[13] This hydroxylation has been considered radical rather than electrophilic in character.[57,57a] All these mechanistic and conformational considerations on the level of free proline, however, become a secondary point, if one considers that it is a microsomal RNA-bound polypeptide of considerable size[58] in which selected proline residues are stereospecifically hydroxylated in positions *trans* to the C-2 carboxyl function with full retention of the stereochemistry of C-4.

(49) F. Irreverre, K. Morita, A. V. Robertson, and B. Witkop, *J. Am. Chem. Soc.*, **85**, 2824 (1963).
(50) *Cf.* A. Kaplan, B. Witkop, and S. Udenfriend, *J. Biol. Chem.*, **239**, 2559 (1964).
(51) J. R. Durland and H. Adkins, *J. Am. Chem. Soc.*, **61**, 429 (1939).
(52) R. Criegee, *Ber.*, **77**, 722 (1944).
(53) R. J. Abraham and K. A. McLauchlan, *Mol. Phys.*, **5**, 513 (1962).
(54) M. Chvapil and J. Hurych, *Nature*, **184**, 1145 (1959).
(55) H. Wieland, "On the Mechanism of Oxidation," "Silliman Memorial Lectures," Vol. XXII, Yale University Press, New Haven, Conn., 1932, p. 86.
(56) S. Udenfriend, C. T. Clark, J. Axelrod, and B. B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).
(57) R. Breslow and L. N. Lukens, *ibid.*, **235**, 292 (1960).
(57a) NOTE ADDED IN PROOF.—A direct oxygen atom transfer has recently been suggested: G. A. Hamilton, *J. Am. Chem. Soc.*, **86**, 3391 (1964).
(58) B. Peterkofsky and S. Udenfriend, *ibid.*, **238**, 3966 (1963); *cf.* R. F. Lyndon and F. C. Steward, *J. Exptl. Bot.*, **14**, 42 (1963).

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Factors Affecting the Competitive Formation of Oxazolines and Dehydroalanines from Serine Derivatives[1]

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Several factors affecting the relative amounts of oxazolines and dehydroalanines formed from N-carbonyl-O-sulfonylserine derivatives were investigated. It was found that N-carbobenzoxy derivatives do not form oxazolines even under very favorable conditions while N-*p*-nitrobenzoyl compounds can form either product. Serine esters yield either product while the amide strongly favors the formation of oxazoline, but the dehydroalanine can be obtained if the N-carbonyl group is carbobenzoxy. Strong bases favor the formation of dehydroalanines. More polar solvents favor the formation of oxazolines. The application of these findings to the reactions of sulfonyl proteins is discussed.

Introduction

In the reaction of sulfonyl chlorides with proteins numerous sulfonyl groups may be readily introduced into the protein molecule. One protein site in chymotrypsin reacts faster than the others.[2] With sulfonyl fluorides the reaction is more specific and in the case of esterases only one group reacts readily. This group is introduced at the active site and the resulting sulfonyl enzyme derivative is analogous to the normal acyl enzyme formed during the hydrolysis of substrates.[3,4] The sulfonyl enzyme is inactive, of course, but activity can be restored by suitable nucleophilic agents in the case of acetylcholinesterase or by suitable changes in the medium in the case of chymotrypsin. Sulfonyl fluorides in their reaction with esterases behave quite similarly to the organophosphorus antiesterases and it would therefore be expected that serine is the amino acid residue that is sulfonylated. The reactions of O-substituted serine derivatives are therefore quite pertinent for inferring the chemical possibilities of sulfonylated esterases. The ability of some of these compounds to react to form oxazolines and the isomeric dehydroalanine derivatives is especially interesting. The reactions which are pertinent to the problem are indicated in Chart I.

Working with N-benzoyl-DL-serine methyl ester and thionyl chloride, Fry[5], using the method of Bergmann and co-workers,[6] obtained a "complex salt" with a composition approximating the O-chlorosulfinyl derivative. He prepared the oxazoline from this complex salt and also obtained the oxazoline and dehydroalanine from the chloro compound. Riley[7] obtained the dehydroalanine derivative from N-carbobenzoxy-O-diphenylphosphoryl-DL-serine ethyl ester. Attenburrow[8] prepared the oxazoline from an O-tosylthreonine derivative. Photaki's work[9] is most pertinent for our problem. She obtained *only* the dehydroalanine derivatives from N-carbobenzoxy-O-tosyl-L-serine methyl ester.

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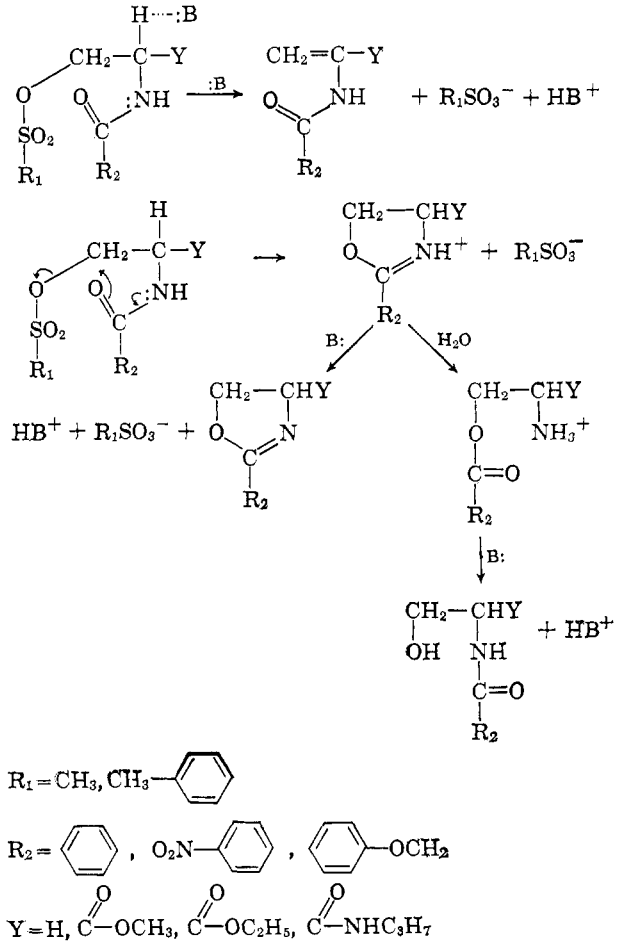
(2) V. Massey, W. F. Harrington, and B. S. Hartley, *Discussions Faraday Soc.*, **20**, 24 (1955).
(3) R. Kitz and I. B. Wilson, *J. Biol. Chem.*, **237**, 3245 (1962); J. Alexander, I. B. Wilson, and R. Kitz, *ibid.*, **238**, 741 (1963).
(4) A. M. Gold and D. Fahrney, *Biochemistry*, **3**, 783 (1964).
(5) E. M. Fry, *J. Org. Chem.*, **14**, 887 (1949).
(6) M. Bergmann and A. Miekeley, *Z. Physiol. Chem.*, **140**, 128 (1924); **143**, 108 (1925); M. Bergmann and E. Brand, *Ber.*, **56**, 1280 (1923).
(7) G. Riley, J. Turnbull, and W. Wilson, *J. Chem. Soc.*, 1373 (1957).
(8) J. Attenburrow, D. F. Elliott, and G. F. Remy, *ibid.*, 310 (1948).
(9) I. Photaki, *J. Am. Chem. Soc.*, **85**, 1123 (1963).



In work with chymotrypsin, Fahrney and Gold[4] suggested that an oxazoline was formed from the sulfonyl enzyme. Under different conditions Strumeyer, White, and Koshland[10] obtained the dehydroalanine derivative of chymotrypsin.

We have studied the reactions of O-tosyl and O-mesyl esters of N-acylserine derivatives with the purpose of finding out if oxazolines can be formed and, if so, what the conditions are that determine whether these products or the dehydroalanines will be obtained. We do, in fact, obtain both oxazolines and dehydroalanines and it is possible to delineate and explain the effect of structural features and reaction conditions in determining the products.

Results and Discussion

The β-elimination reaction with serine derivatives is base-catalyzed and occurs only because the α-hydrogen is acidic. With ethanolamine derivatives, Y = H, the corresponding hydrogen is not sufficiently acidic and only oxazolines are obtained even with strong bases. The formation of an oxazoline is an intramolecular substitution reaction; the carbonyl oxygen is the nucleophile. Even though bases are often necessary for preparing oxazolines, this reaction is not catalyzed by weak bases although it is catalyzed by strong bases.[11] The need for a base arises from the ease with which oxazoline salts hydrolyze even when only small amounts of water are present. In fact we obtained the O-acyl ester which is derived from the oxazoline in the absence of added base and also in the presence of added acetic acid and toluenesulfonic acid, starting with N-*p*-nitrobenzoyl-O-tosylserine ester.

Evidently there are a number of structural features which can be expected to influence the relative amounts of oxazoline and dehydroalanine formed in this situation where substitution and elimination reactions compete.

The effect of the leaving group on the rate of β-elimination reactions in a number of instances roughly parallels its effect in nucleophilic substitution reactions. On this basis one would expect a number of leaving groups to have only a modest effect in determining the ratio of products. When the leaving group is restricted to sulfonates, surely little difference would be expected for different groups, and we found little difference between the methanesulfonate and *p*-toluenesulfonate (Table I). There is scant data from which to draw a general conclusion concerning the effect of the leaving group but it would seem significant that even with a compound having a relatively poor leaving group, chloro, Fry obtained both products, although under different experimental conditions.

Certain chemical structures emerge as strong determinants in controlling the ratio of the products. We find that in contrast to the ethyl ester the propylamide has a very strong tendency to yield only the oxazoline. The underlying consideration is the acidity of the α-hydrogen atom which is weaker in amides than in esters.[12] The acidity of this hydrogen is enhanced by hyperconjugative resonance with the carbonyl group. This kind of resonance is less prominent in amides because in amides resonance of the carbonyl group with the amino nitrogen is more extensive than resonance with the ether oxygen in esters, as indicated by the higher resonance energy of amides. While the ethyl ester of N-*p*-nitrobenzoyl-O-tosylserine readily yields both products, the propylamide yields only the oxazoline even under external conditions which favor the dehydroalanine. Similarly we obtained only the oxazoline from N-phenoxyacetyl-O-tosylserine propylamide. In a paper appearing while this work was in progress Benoiton, Hanson, and Rydon[13] prepared the oxazoline from N-hippuryl-O-tosylserine amide, but obtained the dehydroalanine from the ester.

We find also that N-carbobenzoxyamino alcohols in contrast to N-acylamino alcohols do not form oxazolines. Even with N-carbobenzoxy-O-tosylethanolamine (Y = H), where there is no competition with an elimination reaction, no oxazoline is formed and the starting material is recovered. There is no instance reported in the literature where an N-carbobenzoxy derivative has yielded an oxazoline. The tendency of amides to form oxazoline is completely offset if the N-carbonyl group is carbobenzoxy; N-carbobenzoxy-O-tosylserinepropylamide yielded an unsaturated compound which took up 80% of the expected amount of Br_2. No oxazoline was obtained. Photaki obtained the dehydroalanine from a N-carbobenzoxy-O-tosylserineamide derivative.

(10) D. H. Strumeyer, W. N. White, and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U. S.*, **50**, 931 (1963).

(11) G. L. Schmir and C. Zioudrou, *Biochemistry*, **2**, 1305 (1963); C. Zioudrou and G. L. Schmir, *J. Am. Chem. Soc.*, **85**, 3258 (1963).

(12) R. G. Pearson and R. L. Dillon, *ibid.*, **75**, 2439 (1953).

(13) L. Benoiton, R. W. Hanson, and H. N. Rydon, *J. Chem. Soc.*, 821 (1964).

Base strength is an important determinant; stronger bases increase the amount of dehydroalanine (Table I). Evidently base catalysis is more important in the elimination reaction than in oxazoline formation.

TABLE I[a]

Sulfonic ester	Solvent	Base	Temp., °C.	Oxazoline/dehydroalanine
IIa	Aq. methanol, 70%	Potassium acetate	75	3.0
IIa	Methanol[b]	Potassium acetate	65	0.82
IIb	Methanol	Potassium acetate	65	0.67
IIa	Acetone + methanol, 2:1	Potassium acetate	60	0.33
IIb	Acetone + methanol, 2:1	Potassium acetate	60	0.2
IIb	Pyridine	Pyridine	20, 3 days	0.15
IIa	Pyridine	Pyridine	20, 6 days	+
IIa	Ethanol	Sodium ethylate	78	0.0
IIa	Acetone	Diethylamine[d]	20	0.0
IIb	Acetone	Diethylamine	20	0.0
IIc	Acetone	Diethylamine	56	0.0
IIa	Methanol	Diethylamine	65	+

[a] Oxazoline and dehydroalanine IV and V were separated for approximate quantitative estimation as described under their preparation. Total yields of the two compounds were 80–95%. [b] Similar results with ethanol, but no quantitative estimation was made. [c] Mostly dehydroalanine. [d] Propylamine and triethylamine gave the same results.

There is also an interesting solvent effect (Table I), the more polar solvents favoring the substitution reaction over the elimination reaction. This effect is known from other reactions and is readily explained for the more usual situation in which a negatively charged base is involved in both elimination and substitution.[14] The explanation involves the slowing of both reactions but especially the elimination reaction in more polar solvents. In our case while the base, acetate ion, is negatively charged, the nucleophile in the substitution reaction is uncharged in the presence of a relatively weak base. In this case the transition state for the substitution reaction is more polar than the starting material and the same explanation predicts that the rate of formation of oxazoline is actually increased.

Returning to the literature already cited, we see that Bergmann's procedure, weak base and highly polar solvent, favors the oxazoline, which he in fact obtained. With strong base Fry obtained the dehydroalanine. In Photaki's work only carbobenzoxy derivatives were used and only dehydroalanines were obtained. This agrees with our finding that carbobenzoxy derivatives do not form oxazolines. Our findings and those of others can be summarized as follows: (1) the nature of the leaving group probably is not decisive in determining which product will be obtained; (2) N-carbobenzoxyserine derivatives do not yield oxazolines but do react to form dehydroalanines; (3) with N-acyl groups either product may be obtained; serine esters may yield either product; (4) serine amides very strongly favor the formation of oxazolines, but dehydroalanines can be obtained if oxazoline formation is suppressed as, for example, in the case of carbobenzoxy derivatives; (5) strong base favors formation of dehydroalanines; (6) polar solvents favor the formation of oxazolines.

We now consider the situation with sulfonyl enzyme derivatives in the light of these generalizations. In proteins we have an amide and an acyl group and the medium is highly polar. These factors so strongly favor the formation of the oxazoline that it is unlikely that the dehydroalanine could ever be obtained in a competitive situation. But a competitive situation may not prevail in proteins. It is generally thought that many of the carbonyl groups are held in a hydrogen-bonded condition in proteins and if this should be the case for the acyl group in question, the sulfonyl enzymes would be stable and an oxazoline would not form unless the acyl group were to be freed by a change in environmental conditions. The sulfonyl enzyme derivatives of chymotrypsin and cholinesterase are stable. In urea solution the sulfonyl group is labile. The interpretation that an oxazoline is formed[4] is quite consistent with our studies. The sulfonyl group is also removed by cold sodium hydroxide solution. In this case a dehydroalanine-containing protein is formed.[10] Here to be consistent with our studies we should have to assume that oxazoline formation is suppressed. This may well be the case, however, because chymotrypsin treated with cold sodium hydroxide solution does not loose its catalytic activity.[10] It is possible, therefore, that the acyl group is still held in a nonreactive position.

The N-carbobenzoxydehydroalanine derivatives absorb in the ultraviolet in methanol solution at λ_{max} 244 mμ (E_{max} 5.6–6.0 $\times$ 10^3) as reported by Photaki. The 2-phenyloxazolines also absorb at about the same wave length, λ_{max} 242 mμ (E_{max} 0.8–1.2 $\times$ 10^4, as do the oxazoline salts. The spectra of the *p*-nitrophenyl derivatives of both compounds appear to be dominated by that chromophore with λ_{max} 265 mμ (E_{max} 1.2 $\times$ 10^4). The oxazolines show a strong absorption in the infrared (in chloroform solution) at 6.05 μ (1650 cm.$^{-1}$) as previously noted.[15]

Experimental

All compounds were DL-serine derivatives and referred therefore in the text simply as serine. The melting points were uncorrected. Five types of compounds were prepared, three of them according to methods A, B, and C. The variations in method are indicated in the tables.

***N*-*p*-Nitrobenzoylserine Ethyl Ester (I). Method A.**—Serine ethyl ester hydrochloride was nitrobenzoylated by the method of Schotten–Baumann in a two-phase water–chloroform mixture in the presence of sodium bicarbonate.[16] After evaporation of the chloroform layer, the product was recovered and purified by recrystallization from benzene.

N-*p*-Nitrobenzoyl-O-tosylserine Ethyl Ester (IIa). Method B.—The method of tosylation was the same as used by Boyd, *et al.*,[17] and Photaki.[9] To a solution of 5.64 g. (0.02 mole) of I in 18 ml. of pyridine cooled in ice was added 4.2 g. (0.022 mole) of tosyl chloride. After being kept in ice for 30 min. the mixture was brought to room temperature and 100 ml. of ice–water was added with vigorous stirring. The oil solidified very soon, was filtered off, and without drying recrystallized from acetone and then from methanol.

From the acetone mother liquors the O-acyl ester tosylate resulting from rearrangement and hydrolysis of the O-tosyl compound could be recovered. From the aqueous pyridine filtrate a considerable amount of starting material (12%), possibly originating also from rearrangement, hydrolysis, and O→N shift was recovered.

Serine *n*-Propylamide Hydrochloride.—Serine methyl ester hydrochloride (15.6 g., 0.1 mole) was dissolved in 25 ml. (about 0.3 mole) of *n*-propylamine and left at room temperature for several

(14) C. K. Ingold, "Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953.

(15) Thompson, *et al.*, "The Chemistry of Penicillin," Princeton University Press, 1940, p. 382.

(16) R. Ratouis and R. Behar, *Bull. soc. chim. France*, 1258 (1957).

(17) N. Boyd and R. H. Hauser, *J. Am. Chem. Soc.*, **75**, 5896 (1953); N. Boyd and R. C. Rittner, *ibid.*, **82**, 2032 (1960).

TABLE II

N-SUBSTITUTED ETHANOLAMINES AND SERINES AND THEIR O-SULFONIC ACID ESTERS

$R_2CONHCHYCH_2X$

Compd.	R_2	Y	X	Method	Yield, %	M.p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Sulfur Calcd.	Sulfur Found	Ref.
I	$O_2NC_6H_4$	$COOC_2H_5$	OH	A	90	107	51.06	51.00	5.00	5.16	9.93	9.94	..	..	
IIa	$O_2NC_6H_4$	$COOC_2H_5$	$OSO_2C_6H_4CH_3$	B	52	129	52.29	52.06	4.62	4.84	6.42	6.59	7.35	7.40	
IIb	$O_2NC_6H_4$	$COOC_2H_5$	OSO_2CH_3	B	80	121	43.33	43.35	4.48	4.35	7.78	7.89	8.90	9.05	*a*
IIc	$O_2NC_6H_4$	$COOC_2H_5$	Cl	..	85	135	47.93	47.95	4.36	4.43	9.32	9.42	..	..	*b*
VII	$O_2NC_6H_4$	$CONHC_3H_7$	OH	A	62	173	52.87	52.72	5.80	5.83	14.23	14.49	..	..	*c, d*
VIII	$O_2NC_6H_4$	$CONHC_3H_7$	$OSO_2C_6H_4CH_3$	B	45	115	53.44	53.04	5.16	5.30	9.35	8.89	7.13	6.67	*e, f*
XI	C_6H_5	$COOCH_3$	$OSO_2C_6H_4CH_3$	B	..	...									*g, h*
XIV	$C_6H_5OCH_2$	H	$OSO_2C_6H_4CH_3$	B	60	70	58.44	58.11	5.48	5.48	4.01	3.89	9.18	9.13	*i*
XVI	$C_6H_5OCH_2$	$CONHC_3H_7$	OH	A	70	118	59.98	59.87	7.19	7.19	10.00	9.75	..	..	*c, d, j*
XVII	$C_6H_5OCH_2$	$CONHC_3H_7$	$OSO_2C_6H_4CH_3$	B	48	118	58.05	58.60	6.03	6.39	6.45	5.96	7.38	7.45	*i, k*
XX	$C_6H_5CH_2O$	H	$OSO_2C_6H_4CH_3$	B	71	77	58.44	58.17	5.48	5.33	4.01	3.94	9.18	9.25	*l, m*
XXI	$C_6H_5CH_2O$	$CONHC_3H_7$	OH	A	72	111	59.98	60.10	7.19	7.08	10.00	9.99	..	..	*d, j*
XXII	$C_6H_5CH_2O$	$CONHC_3H_7$	$OSO_2C_6H_4CH_3$	B	60	91	58.05	58.14	6.03	6.16	6.45	6.54	7.38	7.38	*i*

[a] Prepared by reaction at room temperature with CH_3SO_2Cl. [b] Prepared according to ref. 5. Intermediate chlorosulfinate was very unstable and gave the chloro derivative on purification. [c] Yield calculated on serine ester HCl. [d] Recrystallized from H_2O. [e] The O-acyl ester tosic acid salt was formed as a by-product. [f] Unstable, rearranges and hydrolyses on standing. [g] Prepared according to ref. 16. [h] Unstable; could not be purified. [i] Recrystallized from methanol. [j] Crude methyl ester treated with 150% excess amine in methanol for 24 hr., excess amine and methanol distilled off *in vacuo*. [k] Reacted at −25°, left at −3° for 15 min. [l] Reference 18. [m] An attempt to rearrange this tosyl derivative to the oxazoline salt or O-ester salt by refluxing in aqueous alcohol failed. Treatment with hot 0.1 *N* sodium methoxide also failed. In both cases the starting material was completely recovered.

TABLE III

O-ACYLSERINE ESTERS AND AMIDES SULFONIC ACID SALTS

$H_3N^+CHYCH_2OCOR_2X^-$

Compd.	R_2	Y	X	Method	M.p., °C.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Sulfur Calcd.	Sulfur Found	Ref.
IIIa	$O_2NC_6H_4$	$COOC_2H_5$	$OSO_2C_6H_4CH_3$	C	179	50.21	50.49	4.88	5.05	6.17	6.10	7.05	7.14	
IIIb	$O_2NC_6H_4$	$COOC_2H_5$	OSO_2CH_3	C	162	41.27	41.21	4.79	4.92	7.41	7.15	8.47	8.16	
IX	$O_2NC_6H_4$	$CONHC_3H_7$	$OSO_2C_6H_4CH_3$	C	211	51.38	51.48	5.39	5.41	8.99	8.61	6.86	6.65	*a*
XIII	C_6H_5	$COOCH_3$	$OSO_2C_6H_4CH_3$	C	159	54.67	54.36	5.35	5.37	3.54	3.69	8.11	8.05	*a, b*
XV	$C_6H_5OCH_2$	H	$OSO_2C_6H_4CH_3$	C	129	55.57	55.53	5.76	5.87	3.81	3.50	8.73	8.10	
XVIII	$C_6H_5OCH_2$	$CONHC_3H_7$	$OSO_2C_6H_4CH_3$	C	148	55.74	55.77	6.24	6.31	6.19	6.30	7.09	7.17	*c*

[a] Formed also from moist crude tosyl ester on standing at room temperature. [b] Prepared from XII by warming in 70% methanol. [c] Purified through repeated precipitation with ether from acetone solutions and several recrystallizations from acetone and ethyl acetate.

days. The resulting brown sirup was mixed with 50 ml. of methanol and 0.1 mole of a methanolic solution (about 4 *N*) of sodium methylate was added. Sodium chloride which precipitated was filtered off and the solution distilled *in vacuo* in order to eliminate the larger part of excess propylamine. The residual sirup was dissolved in water and the pH of the solution adjusted to 3 with dilute HCl. This crude solution was used for the next step (Method A).

O-*p*-Nitrobenzoylserine Ethyl Ester Tosic Acid Salt (IIIa). Method C.—IIa was refluxed for 30 min. in 95% ethanol and the solution evaporated to dryness. The yield was practically 100%. After recrystallization from methanol the salt melted at 179°.

2-*p*-Nitrophenyl-4-carbethoxyoxazoline (IV) and N-*p*-Nitrobenzoyldehydroalanine Ethyl Ester (V).—IIa and IIb gave different proportions of IV and V when treated with different bases. As it is difficult to obtain quantitative separation of the oxazoline base because of its high solubility, it was transformed into its tosic acid salt which in turn hydrolyzed to the corresponding O-acyl ester tosic acid salt, IIIa. The dehydroalanine was not affected and subsequently recovered. A typical procedure was as follows: the variations in solvent, temperature, and reaction time are indicated in Table I.

To a solution of 0.4 g. (0.004 mole) of potassium acetate in 20 ml. of methanol was added 0.87 g. (0.002 mole) of IIa. After refluxing the mixture for 30 min. the solvent was evaporated at low temperature and the residue was washed with water, filtered, and dried at 75°, yielding 0.47 g. (89%). The solid mixture was dissolved in 10 ml. of acetone and 0.34 g. (0.002 mole) of tosic acid was added. After about 5 min. a heavy precipitate of O-ester tosic acid salt was formed. To assure complete precipitation, some absolute ether was added and the product was filtered off, dried, and weighed. The filtrate was evaporated to dryness after addition of sodium bicarbonate solution. The solid was washed with water, filtered, dried, and weighed. Melting points confirmed the identity and purity of the products. The results are given in Table I.

Compound IV (free base) was prepared by fractional crystallization of the crude mixture with dehydroalanine (see above). The oxazoline is the more soluble substance. By discarding the first precipitates from several recrystallizations from aqueous alcohol a pure product was obtained, m.p. 80°. This compound is pure as shown by its quantitative conversion to O-acyl ester tosic acid salt.

N-*p*-Nitrobenzoyldehydroalanine (VI).—V (and also IIa) was treated at room temperature for 10 min. with a dilute aqueous methanolic solution of sodium hydroxide in slight excess. Neutralization of the salt with dilute HCl gave the free dehydroalanine, m.p. 193° (recrystallized twice from acetone).

2-*p*-Nitrophenyl-4-propylcarbaminooxazoline (X).—To a methanolic solution of potassium acetate was added VIII and the mixture refluxed for 1.5 hr. The solvent was then distilled off under reduced pressure and the residue was washed with water, filtered, and dried. The yield was practically quanitative, m.p. 163°, recrystallized from methanol, m.p. 165°.

The same results were obtained in a few minutes using sodium methylate or potassium hydroxide instead of potassium acetate. Little reaction occurred with dimethylamine in acetone at room temperature. However, at boiling temperature a very good yield of oxazoline was obtained.

2-Phenyl-4-carbomethoxyoxazoline Tosic Acid Salt (XII).—The oxazoline was prepared according to the method of Fry.[5] The oil was dissolved in acetone and a calculated amount of tosic

acid was added followed by a small amount of absolute ether. This compound, m.p. 138°, is relatively stable.

2-Phenoxyacetyl-4-propylcarbaminooxazoline (XIX).—Attempts to prepare the oxazoline by the usual method in methanol with potassium acetate or sodium methylate were unsuccessful, possibly because boiling temperature was necessary to dissolve the starting material. However, by changing the solvent from methanol to dimethylformamide, free oxazoline was prepared. To a solution of 4.34 g (0.01 mole) of XVII in 10 ml. of DMF was added 10 ml. of 1 *N* sodium methylate. After standing at room temperature for 10 min. water was added and the product was removed by extraction with ether. Evaporation of the ether left a low melting solid in practically quantitative yield, m.p. 52° after two recrystallizations from ether–petroleum ether.

Addition of the calculated amount of tosic acid to the oxazoline in acetone and subsequent recrystallization of the salt from acetone and ethyl acetate gave the hydrolysis product identical with XVIII. Free oxazoline is unaffected by 1 *N* KOH in aqueous methanol at room temperature.

N-Carbobenzoxydehydroalanine *n*-Propylamide (XXIII).—Reaction of XXII with an equivalent amount of dilute sodium methylate gave very gummy solids in 70% yield. A crystalline solid, m.p. 68°, was obtained after several recrystallizations from ether–petroleum ether. The compound took up 0.8 equiv. of bromine.

Analyses of oxazolines and dehydroalanines are listed in Table IV.

Bromination.—The possible formation of an unsaturated compound was tested by uptake of bromine. The method we used is a modification of standard methods. Less than 40 μmoles of compound was dissolved in 1.0 ml. of glacial acetic acid and 1 ml. of 0.1 *N* $KBrO_3$ (standard) and 0.1 ml. of 4 *N* HCl were added. After 5 min. 200 mg. of KI was added and the iodine was titrated with 0.1 *N* $Na_2S_2O_3$ with starch as an indicator.

TABLE IV

	Analyses, %							
	Carbon		Hydrogen		Nitrogen		Sulfur	
Compd.	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
IV	54.54	54.47	4.58	4.74	10.60	10.99		
V	54.54	54.68	4.58	4.75	10.60	10.46		
VI	50.85	50.55	3.41	3.42	11.86	11.52		
X	56.31	55.91	5.45	5.46	15.16	15.27		
XII	57.28	57.27	5.07	5.17	3.70	3.87	8.50	8.79
XIX	64.10	62.90	6.92	7.02	10.68	10.76		

Bromination helped in the identification of the compounds. N-Carbobenzoxydehydroalanine benzyl ester took up 0.85 equiv. of bromine, and compound V 0.97 equiv. Compound XXIII took up 0.80 equiv. Oxazolines and other compounds did not take up more than 0.05 equiv. All phenoxyacetyl derivatives used up 1.0 equiv. of bromine due to substitution in the ring.

Oxazolines were further identified by the formation of tosic acid salts and/or their hydrolytic products, O-acylserine ester or amide tosic acid salts, as indicated above.

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COMMUNICATIONS TO THE EDITOR

A New Purine Synthesis[1]

Sir:

The condensation of a 4-amino-5-nitroso-pyrimidine with an active methylene compound has become a classical synthetic route to pteridines in those cases where the intermediate anil is capable of intramolecular cyclization by addition of the *o*-amino group to an appropriately situated electrophilic center. Thus, condensation of a 4-amino-5-nitroso-pyrimidine with barbituric acid,[2] cyanoacetic acid,[3] or phenylacetonitrile[4] gives pyrimidopteridines, 7-aminopteridine-6-carboxylic acids, or 6-phenyl-7-aminopteridines, respectively. This reaction has recently been reviewed.[5] It appeared to us that this condensation was potentially capable of giving purines rather than pteridines provided that the *ortho*-situated amino group could be induced to add intramolecularly to the anil grouping itself rather than to an electrophilic center attached to the anil carbon. Aromatization to the final purine would then result from elimination or oxidation.

An attractive candidate for the active methylene component appeared to be a quaternized Mannich base, since the requisite intramolecular addition–cyclization reaction would be facilitated by the positively charged anil nitrogen. Furthermore, the terminal aromatization reaction would involve loss of trimethylamine and thus parallel the Hofmann elimination reaction, which proceeds with great facility when leading to an aromatic system.

We wish to describe a new purine synthesis based upon this principle. Thus, condensation of 1,3-dimethyl-4-amino-5-nitrosouracil (**1**) with benzyltrimethylammonium iodide in refluxing dimethylformamide solution resulted in the evolution of trimethylamine and the separation in 31% yield of 8-phenyltheophylline (**2**, R = C_6H_5).

This reaction appears to be equally applicable to other quaternized Mannich bases.[6]

The only nonquaternized Mannich base which was successfully employed in this condensation reaction was gramine. Condensation of this latter compound with **1** in refluxing dimethylformamide solution resulted in evolution of dimethylamine and the separation of the novel 8-(3′-indolyl)theophylline (**2**, R = 3′-indolyl). An attractive mechanism for this facile condensation involves initial elimination of dimethylamine from gramine, perhaps catalyzed by the nitrosopyrimidine, to 3-methyleneindolenine, followed by nucleophilic

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(2) G. M. Timmis, *Nature*, **164**, 139 (1949); G. M. Timmis, U. S. Patent 2,581,889 (Jan. 8, 1952); *Chem. Abstr.*, **46**, 7594 (1954).

(3) T. S. Osdene and G. M. Timmis, *Chem. Ind.* (London), 405 (1954).

(4) R. G. W. Spickett and G. M. Timmis, *J. Chem. Soc.*, 2887 (1954).

(5) T. S. Osdene in "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Ed., Pergamon Press, London, 1964, pp. 65–73.

(6) Additional compounds prepared by this method were **2**, R = 3′-methyl-1′-indolyl, R = 3′,5′-dimethyl-4′-hydroxyphenyl, and R = 3′-methyl-2′-hydroxyphenyl. Satisfactory analytical data were obtained for all compounds reported.