static potencies to all three test organisms were decreased. Solubility in physiological saline was gradually increased with increasing degree of esterification, but there was no sudden increase in solubility as was experienced in the hydroxyethyl esters.

Acknowledgment.—The authors acknowledge assistance as follows: E. M. Humphreys for bacteriostatic assays, L. M. White and G. E. Secor for amide determinations, and M. D. Carter for Van Slyke amino nitrogen analyses.

Summary

The esterification of subtilin with methanol is accompanied by an increase in bacteriostatic activity to *Micrococcus conglomeratus* and to *Streptococcus faecalis* and a decrease in activity to *Staphylococcus aureus*. The reaction when conducted at 0° is highly specific for esterification of carboxyl groups. At 25°, esterification of carboxyl groups is followed by methanolysis of some of the amide groups without splitting of peptide bonds. Greatly increased solubility is attained when the ester content is increased beyond that for complete reaction of the carboxyl groups.

Ethyl esters prepared under conditions comparable to methylation showed generally decreased bacteriostatic activity and no alcoholysis of amide groups. Some of the products show decreased amino nitrogen. Increase in solubility was only slight.

Hydroxyethyl esters of subtilin showed increased bacteriostatic activity toward *M. conglomeratus* and *Strep. faecalis*. Esterification of subtilin in ethylene glycol was not accompanied by loss of amide nitrogen, but some of the products showed a decrease in amino nitrogen. Although the carboxyl groups could not be completely esterified, some of the esters had greatly increased solubility in physiological saline.

Hydroxypropyl esters showed decreased bacteriostatic activity and only moderate increase in solubility. Esters prepared at 25° showed a decrease in amino nitrogen.

ALBANY 6, CALIF. RECEIVED NOVEMBER 3, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BROOKLYN COLLEGE]

2-Lepidyl Substituted Diamines

BY IRVING ALLAN KAYE

In the course of an investigation on the preparation of N-substituted aminoalcohols, it was observed that replacement of a 2-pyridyl group by a 2-lepidyl substituent gave a compound having enhanced antihistaminic activity.[1] It seemed of interest, therefore, to determine what effect a similar replacement of the 2-pyridyl group in N,N - dimethyl - N′ - (2-pyridyl) - N′ - benzylethylenediamine,[2] and in other active 2-pyridyl substituted diamines[3-5] would have upon the histamine antagonistic activity.

The intermediate 2-lepidyl secondary amines were prepared by one of three general procedures. The method of choice, A, was used only in those cases where the N,N-substituted ethylene or propylenediamines were commercially available. Excellent yields of I and II were obtained by heating 2-chlorolepidine with excess alkylene diamine. Whitmore[6] and Huttrer[2] used the same type of reaction with 2-bromopyridine to prepare 2-amino-alkylaminopyridines. Their products were obtained in good yields when an excess of alkylene diamine was used. Their reactions, however, were run in pyridine as solvent, since poor yields were obtained in its absence, and in sealed tubes, since the reaction temperature was 140–160°. In this investigation it was found that the use of a considerable excess of alkylene diamine in the absence of any solvent eliminated the need for pressure vessels.

Methods B and C were used for the preparation of 2-benzylaminolepidine and analogous amines of structures III–XIII. Method B is an application of the procedure of Tschitschibabin[7a-c] as modified by Huttrer, *et al.*,[2] who replaced sodium amide with the more easily handled and commercially available lithium amide. Method C is an extension of the method of Tschitschibabin and Knunjanz[8] who prepared secondary amines by condensing 2-aminopyridine with benzaldehyde using formic acid as solvent and reducing agent. The method seems to be of special importance where the requisite substituted benzaldehydes are more readily available than the corresponding benzyl chlorides.

The tertiary amines were prepared by Methods

(1) Unpublished work of the author, to appear at a later date.

(2) Huttrer, Djerassi, Beears, Mayer and Scholz, THIS JOURNAL, **68**, 1999 (1946).

(3) N,N - Dimethyl - N′ - (*p* - methoxybenzyl) - N′ - (2 - pyridyl) - ethylenediamine; Bovet, Horclois and Walthert, *Compt. rend. soc. biol.*, **138**, 99 (1944); *C. A.*, **39**, 3070 (1945).

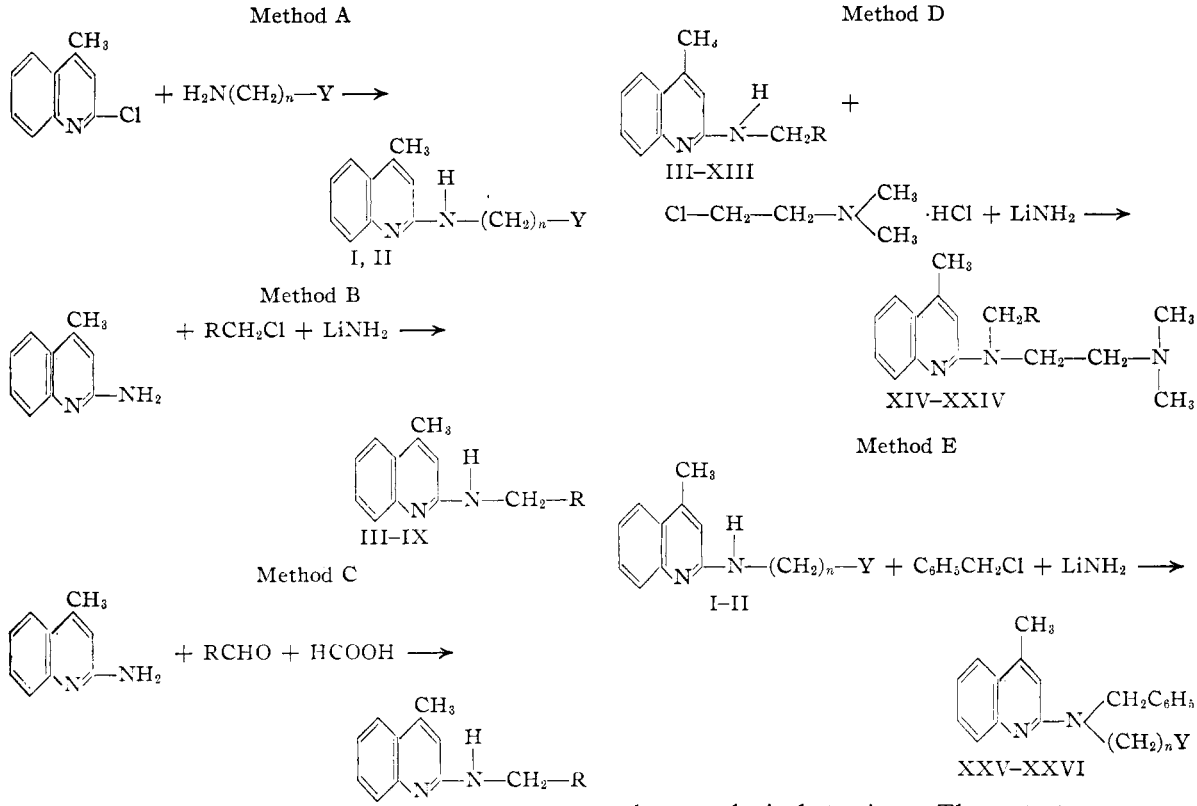
(4) N,N - Dimethyl - N′ - (2 - pyridyl) - N′ - (2 - thenyl) - ethylenediamine: (a) Weston, THIS JOURNAL, **69**, 980 (1947); (b) Ercoli, Schachter, Leonard and Solmssen, *Arch. Biochem.*, **13**, 487 (1947).

(5) N,N - Dimethyl - N′ - (2 - pyridyl) - N′ - (5 - halogeno - 2-thenyl)-ethylenediamine, where the halogens are bromine or chlorine; Clapp, Clark, Vaughan, English and Anderson, THIS JOURNAL, **69**, 1549 (1947).

(6) Whitmore, Mosher, Goldsmith and Rytina, *ibid.*, **67**, 393 (1945).

(7) (a) Tschitschibabin and Seide, *J. Russ. Phys.-Chem. Soc.*, **46**, 1216 (1914); *Chem. Zentr.*, **86**, I, 1064 (1915); (b) Tschitschibabin, Konowalowa and Konowalowa, *Ber.*, **54**, 814 (1921); (c) Tschitschibabin and Knunjanz, *ibid.*, **61**, 2215 (1928).

(8) Tschitschibabin and Knunjanz, *ibid.*, **64**, 2839 (1931).



D and E which differ from Method B and each other only in the nature of the reactants. The secondary amines (I–XIII) were refluxed with excess lithium amide in toluene solution, followed by the addition of β-dimethylaminoethyl chloride hydrochloride (in Method D sufficient lithium amide was added for the liberation of the free base from its salt[2]) or benzyl chloride (Method E). Higher yields were obtained when excess halide, as well as lithium amide, was used. When this was attempted in the preparation of the secondary amines by Method B, poorer yields of products, which analyzed incorrectly, were obtained. The analytical results fell in between the values calculated for III–IX and those for the tertiary amines, C_9H_8N–N–$(CH_2R)_2$. This situation was corrected when equimolecular amounts of amine, lithium amide and halogen compound were used. Since the lithium amide used was of 90–95% purity, the other two reactants were actually used in excess.

Most of the diamines (XIV–XXVI) were purified by redistillation and the free bases were analyzed. Their hydrochlorides were white, crystalline, hygroscopic solids, which were prepared by treating an ethereal solution of the free base with an ethereal hydrogen chloride solution. Since their melting points were not sharp, and since they could not be recrystallized readily, they were prepared under anhydrous conditions from the pure amines and were submitted for pharmacological testing. These tests are currently in progress and will be published elsewhere when completed. Preliminary work has revealed that the activity of some of the compounds in this series is comparable with that shown by the less active compounds presently available.

The author wishes to express his indebtedness to Mr. Max Bart for a systematic literature search of Reaction C and for his help in the preparation of this manuscript, and to Endo Products, Inc., for financial assistance in this project.

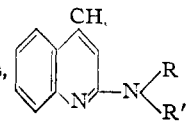
Experimental[9,10]

4-Methylcarbostyril.[11]—This compound was prepared according to the directions, and in amounts 2–5 times greater than those given, in "Organic Syntheses." The crude, dry product, obtained in 90–91% yield, is suitable for the preparation of 2-chlorolepidine.

2-Chlorolepidine.[12]—This compound was prepared according to the directions, and in amounts up to twelve times those given, in "Organic Syntheses." It was found more convenient to filter the crude product, obtained by pouring the hot reaction mixture into a mixture of ice and water, after it had crystallized, and wash the product well with water and aqueous sodium bicarbonate solution, rather than extract with ether. The crude product, after air drying, was obtained in 96% yield. This could be used in the preparation of 2-aminolepidine, but the latter was obtained in a higher state of purity and in better yield when the crude 2-chlorolepidine had been purified by distillation.

(9) All melting points are corrected; boiling points are not.
(10) Microanalyses by Dr. Francine Schwarzkopf.
(11) "Organic Syntheses," **24**, 68 (1944).
(12) *Ibid.*, **24**, 28 (1944).

TABLE I

SECONDARY AND TERTIARY AMINES, 

No.	R	R	Procedure	B. p. °C.	Mm.	M. p., °C.	Yield, %	Nitrogen analyses, % Formula	Calcd.	Found
I	$-CH_2CH_2NC_4H_8O$	H	A	127–144	0.01	280.8–281.6[a]	89	$C_{16}H_{21}N_3O\cdot 2HCl$	20.60[b]	20.37
II	$-CH_2-CH_2-CH_2-NEt_2$	H	A	127	.02[c]	144.0–145.4[d]	98.2[e]	$C_{11}H_{28}N_2$	15.49	15.55
III	$-CH_2C_6H_5$	H	B	156–167	.03	72.4–73.0	74.2	$C_{17}H_{16}N_2\cdot HCl$[f]	12.45[b]	12.58
IV	$-CH_2C_6H_4Cl_2$	H	B	169–170	.03	102.8–103.8[g]	79.7	$C_{17}H_{16}N_2Cl$	9.91	9.90
V	$-CH_2C_6H_4Cl(4)$	H	B			117.6–118.2[h]	49.2[i]	$C_{17}H_{15}N_2Cl$	9.91	10.10
VI	$-CH_2C_6H_3Cl_2(2,4)$	H	B			146.5–147[h]	69.0[i]	$C_{17}H_{14}N_2Cl_2$	8.83	9.05
VII	$-CH_2C_6H_3Cl_2(3,4)$	H	B			103–104[i]	69.0[i]	$C_{17}H_{14}N_2Cl_2$	8.83	8.76
VIII	$-CH_2-2-C_4H_3S$	H	B[k]	163–175	.05	249.5–250[l] (dec.)	61.5	$C_{15}H_{14}N_2S\cdot HCl$	12.19[b]	11.98
IX	$-CH_2-2-(5-Cl-C_4H_2S)$	H	B[m]	171–175	.05	224–226.5[n]	63.2	$C_{15}H_{14}N_2ClS\cdot HCl$	10.87[b]	10.80
X	$-CH_2-C_6H_4(OCH_3)(4)$	H	C	181–187	.05	193–194	62.7	$C_{15}H_{18}N_2O\cdot HCl$	11.26[b]	11.14
XI	$-CH_2-C_6H_3(OCH_3)_2(2,3)$	H	C	198–203	.09	166–168	36.4	$C_{19}H_{20}N_2O_2$	9.09	8.93
XII	$-CH_2-C_6H_3(OCH_3)_2(3,4)$	H	C	203–207	.07	146–147[j]	42.9	$C_{19}H_{20}N_2O_2$	9.09	8.81
XIII	$-CH_2-C_6H_3(O_2CH_2)(3,4)$	H	C	190–192	.06	220–221.5	34.0	$C_{18}H_{16}N_2O\cdot HCl$	10.78[b]	10.61
XIV	$-CH_2C_6H_5$	$-CH_2-CH_2NMe_2$	D[p]	158–165	.06		72.6	$C_{21}H_{28}N_2$	13.16	12.86
XV	$-CH_2C_6H_4Cl(2)$	$-CH_2-CH_2NMe_2$	D	156	.03	214–215[q]	78.8	$C_{21}H_{24}N_2Cl\cdot 2HCl$[q]	16.62[b]	16.18
XVI	$-CH_2C_6H_4Cl(4)$	$-CH_2-CH_2NMe_2$	D	178	.04		88.0	$C_{21}H_{24}N_2Cl\cdot 2HCl$[r]	16.62[b]	16.53
XVII	$-CH_2C_6H_3Cl_2(2,4)$	$-CH_2-CH_2NMe_2$	D	192	.05		85.8	$C_{21}H_{23}N_2Cl_2$	10.82	11.16
XVIII	$-CH_2C_6H_3Cl_2(3,4)$	$-CH_2-CH_2NMe_2$	D	183–187	.03		77.4	$C_{21}H_{23}N_2Cl_2$	10.82	10.98
XIX	$-CH_2-2-C_4H_3S$	$-CH_2-CH_2NMe_2$	D	159–172	.05		74.0	$C_{19}H_{26}N_2S$	12.91	13.09
XX	$-CH_2-2-(5-Cl-C_4H_2S)$	$-CH_2-CH_2NMe_2$	D	161–164	.07		69.5	$C_{19}H_{22}N_2ClS$	11.68	11.38
XXI	$-CH_2C_6H_4(OCH_3)(4)$	$-CH_2-CH_2NMe_2$	D	180	.07		55.0[s]	$C_{22}H_{27}N_2O$	12.03	11.82
XXII	$-CH_2C_6H_3(OCH_3)_2(2,3)$	$-CH_2-CH_2NMe_2$	D	189–191	.08		55.2[s]	$C_{23}H_{32}N_2O_2$	11.07	10.84
XXIII	$-CH_2C_6H_3(OCH_3)(3,4)$	$-CH_2-CH_2NMe_2$	D	192–193	.07		45.0[s]	$C_{23}H_{32}N_2O_2$	11.07	11.81
XXIV	$-CH_2C_6H_3(O_2CH_2)(3\,4)$	$-CH_2-CH_2NMe_2$	D	189–190	.06		46.9[s]	$C_{23}H_{32}N_2O_2$	11.56	11.49
XXV	$-CH_2C_6H_5$	$-CH_2-CH_2NC_4H_8O$	E	196	.05		86.8	$C_{22}H_{27}N_2O$	11.63	11.86
XXVI	$-CH_2C_6H_5$	$-(CH_2)_3-NEt_2$	E	176–182	.05		79.5	$C_{24}H_{31}N_2$	11.62	11.72

[a] Dihydrochloride, recrystallized from absolute ethanol. Further recrystallization from the same solvent lowered the melting point. [b] Ionizable chlorine. [c] Most of the distillations were done in a distillation apparatus similar to that described by Hickman and Weyerts, THIS JOURNAL, 52, 4718 (1930), without the manometer side-arm. [d] Dihydrochloride, recrystallized once from isopropyl alcohol–acetone and again from isopropyl alcohol. [e] The reaction mixture was heated at a bath temperature of 180–189° for seven hours. Longer periods of refluxing gave poorer yields (a 70% yield resulted after refluxing nineteen hours) due apparently to decomposition, evidenced by large amounts of tarry by-products. [f] Recrystallized from ethanol, m. p. 236–238.5°. [g] Recrystallized from ethanol. [h] Recrystallized from benzene. [i] Instead of isolating the compound by distillation, the residue remaining after removal of the ether and toluene, was triturated with petroleum ether. The yield is based on the amount of this crude product. [j] Recrystallized from methanol. [k] The 2-thenyl chloride used in this preparation was prepared by Mr. Louis Silberman of The Brooklyn College Graduate School in 52% yield by the method of Blicke and Burckhalter, THIS JOURNAL, 64, 477 (1942). [l] Hydrochloride; recrystallization from ethanol lowered the melting point. [m] The 5-chloro-2-thenyl chloride was prepared by Mr. Norman A. Rosenthal of The Brooklyn College Graduate School in 44.5% yield. Clapp, *et al.*,[5] report no yield for this compound. [n] Hydrochloride; recrystallized from 95% ethanol. [o] Hydrochloride; recrystallized from ethanol–ether. [p] All the products obtained by methods D and E were viscous yellow oils. [q] This compound was the only one of those obtained by methods D and E which gave a non-hygroscopic hydrochloride. The crude salt, prepared in anhydrous ether, deliquesced, but no longer showed this property after recrystallization. [r] Hygroscopic salt. [s] The yield is of re-distilled product.

2-Aminolepidine.[13]—The method of Jacini, modified to permit an easier isolation and better yield of product, was employed. A vigorous stream of ammonia gas was bubbled through a solution of 80 g. of 2-chlorolepidine in a mixture of 160 g. of phenol and 80 g. of acetamide, maintained at 160–170° for five hours. The reaction was exothermic at first, so that the external heating had to be varied in order to maintain the desired temperature. A white solid, probably ammonium chloride, appeared during the course of the reaction. The phenol and acetamide were then removed by distilling under reduced pressure and the molten residue suspended in ice-water. A saturated aqueous sodium hydroxide solution was added until precipitation seemed complete. After standing a few hours the oil changed to a yellow solid. This was separated by filtration, washed well with water, and recrystallized from benzene. The yield of white crystalline product, m. p. 132–133°, was 57.0 g. (78%). Recrystallized once more from benzene, the compound melted at 133.0–133.3°.[13] Repetition of this reaction, using 3 moles of crude chlorolepidine (not distilled), gave a 76% yield of product, m. p. 121–128°. A later run with 4.5 moles of crude lepidine gave only a 64% yield, m. p. 121–124.5°.

The hydrochloride, prepared by treating a filtered ethereal solution of the base with ethereal hydrogen chloride solution, recrystallized twice from isopropyl alcohol and dried in an Abderhalden pistol at 100° under 0.1 mm. pressure, melted at 201–202°.

Anal. Calcd. for $C_{10}H_{10}N_2\cdot HCl$: Cl, 18.26. Found: Cl, 18.22; 18.28.

The acetyl derivative was prepared by refluxing a solution of 130 g. of 2-aminolepidine in 500 ml. of acetic anhydride for two and one-quarter hours. The acetic anhydride and acetic acid were removed *in vacuo*. The residue, treated with 100 ml. of cold methanol, deposited a white precipitate. This was removed by filtration and washed with cold methanol. The crude product weighed 147.6 g. (90%). Recrystallized twice from ethanol, the compound melted at 230.6–231.8°.

Method A. 2-(β-Morpholinoethyl)-aminolepidine (I).[14]—A mixture of 106.6 g. of 2-chlorolepidine (0.6

(13) Jacini, *Gazz. chim. ital.*, **70**, 621 (1940); *C. A.*, **35**, 3254 (1941). Jacini obtained a 50% yield of product, m. p. 130°.

(14) Krahler and Burger, THIS JOURNAL, **63**, 2369 (1941), prepared this compound as the dihydrochloride, m. p. 272–273°, but did not report their yield.

mole) and 312.0 g. (2.4 moles) of aminoethylmorpholine[15] was heated at a bath temperature of 175–180° for thirty-eight and one-half hours. The cooled reaction mixture was suspended in about 2–2.5 liters of ether. The hygroscopic precipitate was separated by filtration and washed well with ether. The filtrate, after removal of the ether and excess aminoethylmorpholine, was distilled *in vacuo*. The product, a viscous yellow liquid, b. p. 127–144° at 0.01 mm.[16] was obtained in 89% yield (144.7 g.).

Method B. 2-Benzylaminolepidine (III).—A mixture of 31.6 g. (0.2 mole) of 2-aminolepidine and 4.6 g. of lithium amide[17] in 100 ml. of dry toluene[18] was refluxed for two hours in an oil-bath at 120–130°. After cooling somewhat, 25.3 g. (0.2 mole) of benzyl chloride in 50 ml. of dry toluene was added and the mixture refluxed twenty-one and one-half hours longer. The mixture was filtered and the precipitate washed well with ether. After removal of the ether and toluene from the filtrate, the residue was distilled *in vacuo*, yielding 36.9 g. (74.2%) of a viscous orange oil, b. p. 156–167° at 0.03 mm.[16] The product solidified on rubbing with petroleum ether, m. p. 72.4–73.0°.

Method C. 2-(4-Methoxybenzyl)-aminolepidine (X).—A solution of 31.6 g. (0.2 mole) of 2-aminolepidine and 27.2 g. (0.2 mole) of anisaldehyde in 50 ml. of formic acid (practical grade, 85–90%) was refluxed for sixteen days.[19] Water and ice were added to the cooled solution which was then made alkaline and extracted with chloroform. After drying over anhydrous potassium carbonate and removing the solvent, the residue was distilled under reduced pressure. The viscous yellow oil weighed 35.0 g. (62.7%) and distilled at 181–187° at 0.05 mm.[16]

Method D.—Since compounds XIV–XXIV were all prepared in exactly the same way, only the general method is described, and the results summarized in Table I. A mixture of 0.04 mole of the secondary amine, III–XIII, 2.5 g. of lithium amide and 100 ml. of dry toluene was refluxed for two hours. After cooling somewhat, 7.5 g. (0.052 mole) of β-dimethylaminoethyl chloride hydrochloride followed by 50 ml. of toluene were added and the reaction mixture refluxed an additional nineteen to twenty hours. After removing the lithium chloride and solvent (as in method B), the residue was distilled *in vacuo*.

Method E.—This method is the same as the previous except for the amounts of reactants, which were changed to include 0.05 mole of the diamine (I or II), 1.6 g. of lithium amide and 8.2 g. (0.065 mole) of benzyl chloride. The results are summarized in Table I.

(15) The author is indebted to the Sharples Chemical Co. for samples of several of their products.

(16) Since the product was distilled in a Claisen flask with a wide side-arm, the actual distillation pressure was much higher.

(17) Purchased from Metalloys Corp., Minneapolis, Minn.

(18) The toluene was dried over calcium hydride, a sample of which had been generously contributed by the Metal Hydrides Co., Inc.

(19) This is probably not the optimum length of time for this particular reaction. In a model experiment, using benzaldehyde, a 21.1% yield of crude product, isolated as the hydrochloride, was obtained after a nineteen hours' reflux period. The yield rose to 37.2% when the refluxing was extended over a sixteen-day period. In the case of some of the substituted benzaldehydes, better yields might be expected with less tarry by-products if the reflux period were shortened.

Summary

2-Benzyl-, substituted benzyl-, thenyl- and substituted thenyl-aminolepidines were prepared by treating 2-aminolepidine with the appropriate alkyl halide in the presence of lithium amide, or with an aldehyde in the presence of formic acid. 2-Lepidylaminoalkylamines were prepared by refluxing 2-chlorolepidine in excess of the alkylene diamine. The former products were then condensed with β-dimethylaminoethyl chloride hydrochloride in the presence of lithium amide and the latter similarly treated with benzyl chloride. These N,N-disubstituted-2-lepidylamines were prepared as possible antihistaminic agents.

BROOKLYN 10, N. Y. RECEIVED FEBRUARY 2, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY OF THE UNIVERSITY OF CHICAGO AND THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

3α,12α-Dihydroxy-etiocholan-16-one[1a]

BY CHARLES W. MARSHALL[1b] AND T. F. GALLAGHER

In a previous communication[2] it was reported that ozonization of the enol acetate 3α,12α,20-triacetoxy-Δ^{17}-pregnene (I) yielded, in addition to the anticipated 17-ketosteroid, a second crystalline product melting at 219–220.5°; $[\alpha]^{25}D$ + 40° (chloroform). The product showed an absorption spectrum in the ultraviolet with a maximum at 2490 Å. and the elementary analysis was consistent with the formula $C_{27}H_{38}O_7$. These facts are best explained on the assumption that oxidation by ozone had converted the reactive methylene group at C-16 to a ketone without rupture of the unsaturated bond from C-17 to C-20. A molecular model of (I) demonstrates that the two acetoxy groups at C-20 and C-12 together with the angular methyl group at C-13 can very effectively screen the olefinic linkage between C-17 and C-20, so that these two carbon atoms are almost completely shielded by the substituent groups. It is not surprising under these circumstances that ozone attacks C-16 with the formation of the α,β-unsaturated ketone (II). It is well known that the rate of ozonide formation is relatively slow[3,4] with heavily substituted olefins; the persistence of II in the presence of excess ozone is somewhat unexpected but is probably to be explained by the steric factors which prevented the ozonolysis of the olefinic bond initially.

The absorption spectrum of II is especially

(1) (a) The work described in this paper was supported in part by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council. (b) Present address: Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.

(2) Marshall, Kritchevsky, Lieberman and Gallagher, THIS JOURNAL, **70**, 1837 (1948).

(3) Noller, Carson, Martin and Hawkins, *ibid.*, **58**, 24 (1938).

(4) Harries, *Ber.*, **36**, 1933 (1903).