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Jervine. X. Quaternary Dihydrometoxazine Salts as Intermediates in the Jervisine Rearrangement

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N-Acetyljervine, $C_{29}H_{41}O_4N$, on treatment with methanolic hydrogen chloride forms in addition to the known N-acetylisojervine a quaternary chloride $C_{29}H_{42}O_4NCl$ which on removal of the anion with alkali carbonate immediately rearranges to the tertiary base VIII (now named jervisine 17-monoacetate) previously obtained by alkaline hydrolysis of the acetolysis product V.[1] The dihydro-1,3-oxazine structure X assigned to this salt rests on its conversion by catalytic reduction to the hydrochloride XII of the alkali- and acid-stable tertiary tetrahydro-1,3-oxazine base XIII, and on the fact that the latter's 3,23-diacetyl derivative XIV on sulfuric acid-catalyzed acetolysis afforded acetaldehyde and the secondary base 3,23-diacetyl-11-ketoveratramine (III), evidently *via* the aldehyde–ammonia IV. The previously described[1] perchlorate formed in the perchloric acid-catalyzed acetolysis of diacetyljervine, which on treatment with weak alkali rearranges to jervisine triacetate (IX), must accordingly be written as XI. Mechanisms for the formation of these quaternary salts and their rearrangement to jervisine derivatives are proposed.

As reported in an earlier paper of this series,[1] the sulfuric acid-catalyzed acetolysis of diacetyljervine (I) leads to two products: the indanone II (triacetyl-11-ketoveratramine[2]) and the "open" triacetate V, which may be a precursor of II.[1] On treatment with cold methanolic potassium hydroxide the indanone II merely suffers hydrolysis of its 3- and 23-acetoxy groups, so that the product is the corresponding N-acetyl derivative. In the case of the triacetate, V, however, the O-desacetylation is accompanied by a rearrangement in which the N-acetyl group migrates to the tertiary hydroxyl at C-17 and the now basic nitrogen forms a covalent bond with carbon atom 17a at the terminus of the α,β-unsaturated keto system.[1] The resulting weak tertiary base VIII on acetylation in pyridine yielded the 3,17,23-triacetate IX, isomeric with V. To facilitate reference to these rearranged products we have assigned the name jervisine to the unacetylated parent base; accordingly VIII will henceforth be called jervisine 17-monoacetate, and IX, jervisine triacetate.

In the same paper[1] we reported that jervisine triacetate (IX) can be obtained more conveniently *via* a perchlorate salt, m.p. 220–222°, which is formed in good yield when diacetyljervine (I) is acetolyzed with acetic anhydride and acetic acid containing 2 molar equivalents of perchloric acid. This salt, which still shows α,β-unsaturated ketone absorption though with characteristics somewhat different from those of jervine (λ_{max} 243 mμ, 16,400; 365 mμ, 74; jervine: λ_{max} 250 mμ, 15,000; 360 mμ 70) was found to rearrange with extraordinary ease to jervisine triacetate (IX) merely when brought in contact with weak bases such as sodium bicarbonate or pyridine. The salt analyzed well for $C_{33}H_{45}O_6N\cdot HClO_4$, and this was difficult to rationalize for the following reason: The rearrangement to jervisine triacetate showed that 3 acetyl groups were present, but these obviously had to be all O-bound, since the nitrogen atom was now basic. However, a secondary base with 3 acetoxy groups (for instance VI, which could have arisen from V by N→O acetyl migration) requires the composition $C_{33}H_{47}O_7N\cdot HClO_4$ with one more H_2O in the molecule. Indeed, structure VI specifically seemed to be favored by the following facts: (1) the triacetate V on treatment with the perchloric acid-containing acetolysis mixture afforded the perchlorate in good yield, and (2) the infrared spectrum of the salt showed a band at 2.98 μ[3] which appeared to originate in >NH, as a similar band was exhibited by piperidine perchlorate but not by potassium perchlorate.

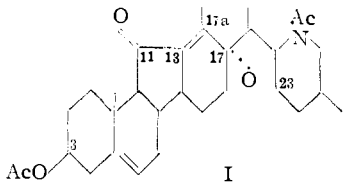
New evidence has now come to hand from an unrelated line of experimentation which shows quite clearly that the perchlorate is in reality derived from a cyclic anhydro base having structure XI. In the course of a study reported in paper XI of this series[4] we had occasion to isomerize N-acetyljervine to N-acetylisojervine with methanolic hydrogen chloride, a reaction which we had first carried out on a small scale in 1950. At that time two by-products were obtained in small amounts which to judge from their absorption spectra were not related to isojervine and hence were set aside. The first of these has now been identified as jervisine 17-monoacetate (VIII). The second substance contained chlorine, then thought to be organically bound but now found to be present as chloride ion. A re-determination of its ultraviolet spectrum revealed complete identity with that of the perchlorate discussed above. It could then be presumed that the base component of the chloride differed from that of the perchlorate only by the absence of the 3- and 23-acetyl groups, and that the salt was the precursor of the jervisine monoacetate accompanying it. Indeed, when the work-up procedure was conducted under acidic conditions throughout, the chloride was obtained in 30–40% yield; on subsequent treatment with sodium carbonate it rearranged quantitatively to VIII. The analysis of the pure salt (m.p. 246–250°) left no doubt that its basic component was isomeric with the starting product, N-acetyljervine, and this, taken in conjunction with the analytical and infrared evidence for the absence of O-acetyl, could only mean that the oxidic ring E of jervine had been replaced by a new ring made up in part of the N-acetyl moiety. The only expression satisfying this requirement, and at the same time accounting for the reappearance of basic properties, is X, in which that moiety forms part of a 4,5-dihydro-1,3-oxazine

(1) O. Wintersteiner and M. Moore, THIS JOURNAL, **75**, 4938 (1953).

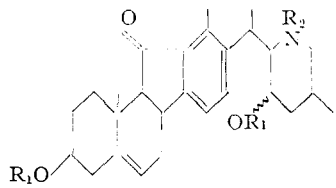
(2) O. Wintersteiner and N. Hosansky, *ibid.*, **74**, 4474 (1952).

(3) More recent measurements with a double beam self-recording instrument (Perkin–Elmer Model 21) have failed to substantiate this finding.

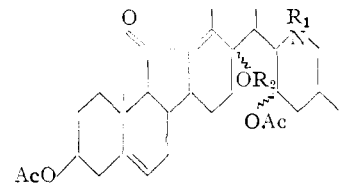
(4) O. Wintersteiner and M. Moore, to be published.



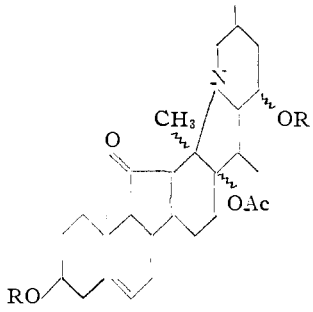
I



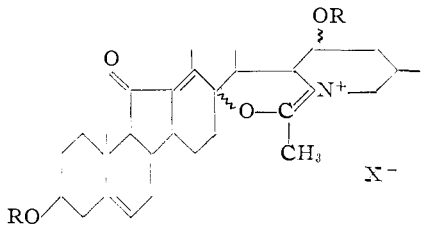
II, $R_1 = R_2 = Ac$
III, $R_1 = Ac$, $R_2 = H$
IV, $R_1 = Ac$, $R_2 = CH(OAc)CH_3$



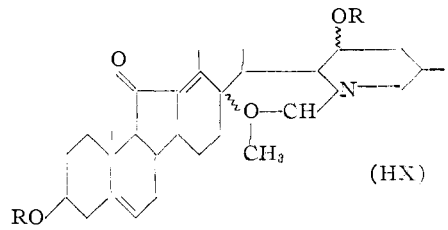
V, $R_1 = Ac$, $R_2 = H$
VI, $R_1 = H$, $R_2 = Ac$
VII, $R_1 = Ac$, $R_2 = SO_3H$



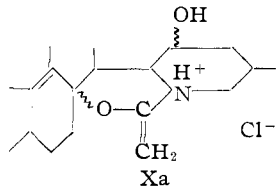
VIII, R = H
IX, R = Ac



X, R = H, X = Cl
XI, R = Ac, X = ClO_4



XIII, R = H; XII, hydrochloride
XIV, R = Ac; XV, hydrochloride;
XVI, perchlorate



Xa

ring. Originally, consideration was given also to the alternative vinylamine structure Xa, mainly on account of certain anomalies in the infrared data which will be discussed later. To gain clarity on this point we subjected the chloride to ozonolysis. Since no formaldehyde was obtained, the *a priori* much more likely[5,6] quaternary salt structure X seems secure. The perchlorate from the perchloric acid-catalyzed acetolysis of diacetyljervine must accordingly be written as XI.

Quaternary Schiff base salts containing the grouping $>C{=}N^+<$ are known to be readily reducible by catalytic means, and this was also true of X. On hydrogenation in aqueous ethanol with platinum oxide as the catalyst the salt rapidly consumed one mole of hydrogen. The resulting tertiary base hydrochloride XII contained solvent of crystallization which could not be removed by drying without loss of hydrochloric acid, and was therefore converted to the free base XIII and further to the 3,23-diacetylated base XIV, both of which gave satisfactory analyses (XIII for the hemihydrate). The diacetate in turn gave the hydrochloride XV, and the perchlorate XVI, identical with the product obtained by catalytic reduction of the quaternary perchlorate XI.

It is characteristic for simple monocyclic tetrahydro-1,3-oxazines and their salts that they readily dissociate on mild acid hydrolysis, or in some cases merely on standing in aqueous solution, into the aldehyde and the 1,3-amino alcohol from which they are usually prepared.[7] It could then be expected that the above reduced compounds would cleave on acid hydrolysis with equal facility to yield acetaldehyde and secondary amine salts having the "open" structure represented by the acetolysis product V. This, however, was not the case. Thus the hydrochloride XII was recovered unchanged after several hours of refluxing with 5 *N* hydrochloric acid, or even with dinitrophenylhydrazine reagent. Since, as will appear below, the assigned structure is undoubtedly correct, the unprecedented stability to acid of the tetrahydrooxazine ring in these compounds must be ascribed to constitutional factors (fusion with ring F, spirane structure).

As we had been successful in opening the similarly acid-resistant tetrahydrofuran ring of diacetyltetrahydrojervine by sulfuric acid-catalyzed acetolysis,[8] we applied this procedure to the diacetylated base XIV. While the acidic solution resulting from the treatment of the acetolyzed mixture with ice-water gave only a very faint Schiff test, acetaldehyde could be demonstrated readily after alkalinization with sodium carbonate. Chromatography of the chloroform-extractable products yielded in addition to unattacked XIV a crystalline base $C_{31}H_{41}O_5N$, which, since it exhibited the same ultraviolet spectrum as the indanone II and was convertible to this compound by acetylation, must be 3,23-diacetyl-11-ketoveratramine (III). Clearly this base was not present as such in the acetolyzed mixture, but was formed during the work-up procedure from an alkali-labile precursor containing the acetaldehyde moiety linked to the nitrogen atom, *e.g.*, the aldehyde ammonia IV. Various explanations for the origin of the third

(5) N. J. Leonard and V. W. Gash, THIS JOURNAL, **76**, 2781 (1954).

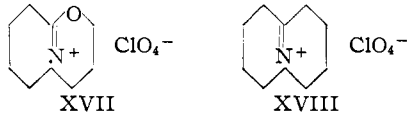
(6) N. J. Leonard, A. S. Hay, R. W. Fulmer and V. W. Gash, *ibid.*, **77**, 439 (1955).

(7) K. Hess and C. Uibrig, *Ber.*, **48**, 1978 (1915); C. Mannik and H. Wieder, *ibid.*, **65**, 385 (1932); E. L. Hirst, J. K. N. Jones, S. Minatian, F. W. Ochynsky, A. T. Thomas and T. Urbansky, *J. Chem. Soc.*, 924 (1947).

(8) O. Wintersteiner, M. Moore and B. M. Iselin, THIS JOURNAL, **76**, 5609 (1954).

double bond in ring D have been advanced in a previous paper.[1]

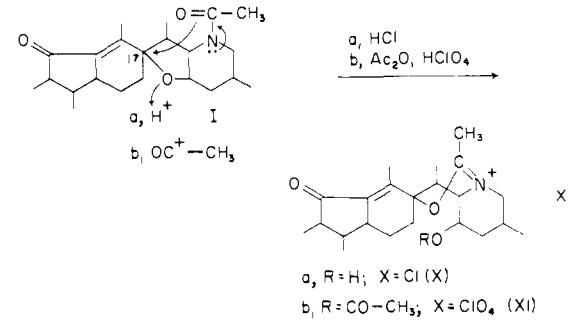
Infrared and Ultraviolet Spectra.—The grouping >C=N⁺< generally gives rise to an infrared band of high intensity in the 5.88–5.94 μ (1639–1665 cm.⁻¹) region.[5,6,9–11] With the quaternary salts X and XI the evaluation of the double bond region for such a band is complicated by the presence of the C=O and C=C bands originating in the α,β-unsaturated keto group. In fact their spectra differ in this region from those of jervine hydrochloride and of the reduced compounds XII and XVI only by a slight to moderate hypsochromic displacement of the C=C band (6.14 → 6.10 to 6.12 μ), and, in the case of X, also of the C=O band (5.88 → 5.82 (broad) μ).[12] At any rate, they do not display an additional intense band in the 5.9–6.0 μ region. However, there was no assurance that the —C=N stretching frequency of the —O—C=N⁺< grouping in the dihydrometoxazine salts would also fall into this narrow range, shown so far to be characteristic only for >C=N⁺< not substituted with oxygen at the carbon. A bicyclic model compound suitable for gaining information on this point, 2,3-cyclohexeno-4,5-dihydro-1,3-oxazine perchlorate (XVII),[13] was kindly made available to us by Professor N. J. Leonard of the University of Illinois. We measured the infrared spectrum of this salt in Nujol and found the —C=N⁺< band to be located at 5.97 μ. Since its Δ¹-piperideine analog, Δ⁵⁽¹⁰⁾-quinolizidinium perchlorate (XVIII), exhibits this band at 5.90 μ,[6] it may be assumed that substitution of the carbon in >C=N⁺< with oxygen generally tends to shift this band to a lower frequency. On this premise the band ~ 6.10 μ may well owe its high intensity to a contribution from this grouping.



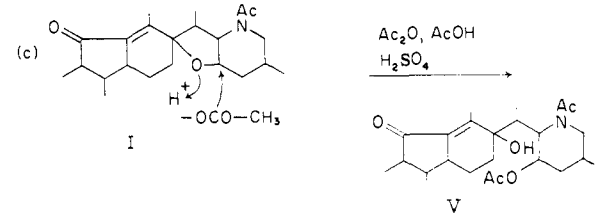
XVII XVIII

The marked modification of the ultraviolet characteristics of jervine consequent to its conversion to the quaternary salts X and XI appears to be due mainly if not entirely to a vicinal effect exerted on the α,β-unsaturated ketone chromophore by the positively charged nitrogen atom, rather than to a contribution to absorption in the 230–250 mμ region by the –O–C=N⁺< grouping (which in any case would not explain the bathochromic shift of the low intensity maximum from 360 to 365 mμ). We conclude this (1) from the observation that the quaternary perchlorate XVII does not absorb radiation above 220 mμ, and (2) from the fact that subtraction of the absorption curve of jervine hydrochloride from that of the quaternary perchlorate XI gives a curve which shows negative extinction values between 250 and 285 mμ reaching ε −3400 at 265 mμ.

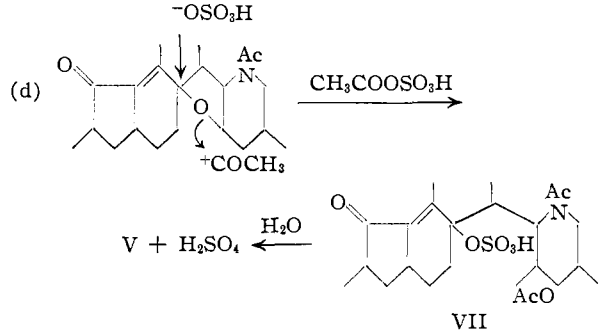
Discussion.—A reasonable mechanism for the formation of the cyclic quaternary Schiff base salts X and XI from N-acetyljervine and diacetyljervine, respectively, would be



For the formation of the acetolysis product V from diacetyljervine we have previously[1] suggested the mechanism below which differs from reactions a and b above in that the oxygen C-23 bond rather



than that to C-17 is broken, and thus provides an explanation for the fact that the 17-hydroxyl function remains unacetylated. However, with the elucidation of the structure of the perchlorate XI this mechanism has become questionable insofar as it is improbable that the nature of the acid used as the catalyst under otherwise identical acetolytic conditions (perchloric acid in b, sulfuric acid in c) could determine as decisively as it does the course of the reaction. A more plausible formulation of reaction c is the one given below (d) which assumes the addition to I of acetylsulfuric acid (the mixed anhydride of acetic acid and sulfuric acid)[14] with the intermediate formation of the 17-sulfuric acid ester VII.



(9) B. Witkop and J. B. Patrick, THIS JOURNAL, **75**, 4474 (1953).

(10) O. E. Edwards, F. M. Clarke and B. Douglas, *Can. J. Chem.*, **32**, 235 (1954).

(11) O. E. Edwards and T. Singh, *ibid.*, **32**, 465 (1954).

(12) It is characteristic of free jervine and other compounds containing the jervine chromophore (*cf.* R. Hirschmann, C. S. Snoddy, Jr., C. F. Hiskey and N. L. Wendler, THIS JOURNAL, **76**, 4013 (1954)), that the C=C band exceeds or at least equals in height the C=O band. However, in the spectrum of the hydrochloride (Nujol) the former band, though still of high intensity, is somethat lower than the carbonyl band. The quaternary salts conform in this respect with jervine base (C=C ≧ C=O), and the reduced compounds with jervine hydrochloride (C=C < C=O).

(13) N. J. Leonard and M. Oki, University of Illinois, unpublished results. We wish to express our sincere thanks to Dr. Leonard for the gift of this compound.

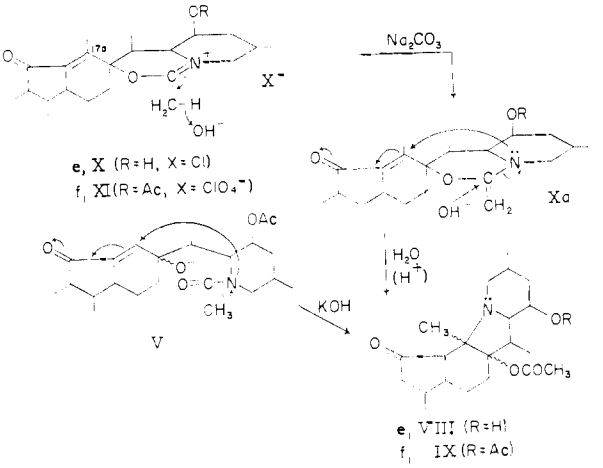
(14) Franchimont, *Compt. rend.*, **92**, 1054 (1881); T. F. Murray and W. O. Kenyon, THIS JOURNAL, **62**, 1230 (1940).

It is likely that attack at C-17 by the $^{-}OSO_3H$ ion would take precedence over that by N-acetyl oxygen in reaction b, so that the product would be VII rather than the quaternary Schiff base sulfate corresponding to the perchlorate XI.

There is some circumstantial evidence supporting this hypothesis. When in one of the acetolysis experiments undertaken in 1952 the concentration of sulfuric acid was decreased to half (0.5 vol. per cent.) that found to give the best yields of II and V,[1] neither of these compounds could be isolated from the acetolyzed material either directly or by chromatographing. The bulk of the material consisted of highly polar fractions containing sulfur which could be liberated as sulfate ion by acid hydrolysis. From one of these fractions an acidic crystalline product (m. p. 208–209°) having the analytical composition required for the sulfuric acid ester VII was obtained in small amounts. The ultraviolet spectrum of this compound was similar to that of the quaternary perchlorate XI (λ_{max} 244 mμ, 15,000), but the possibility of its being the corresponding acid sulfate salt (which is isomeric with VII), is ruled out not only by the absence of free sulfate ion but also by the spectral changes following alkalinization, which were indicative of the formation of the indanone II rather than of jervisine triacetate. As the material on hand was not sufficient for hydrolysis, the question whether this sulfuric acid ester is really the precursor of II and V must be left open at this time. The finding that a much larger amorphous sulfuric ester fraction, which followed the crystalline ester in the chromatogram but differed from it by its ultraviolet characteristics (maxima at 250 and 320 mμ), on acid hydrolysis gave a new substance apparently isomeric with diacetyljervine suggests the need for caution and a more detailed study of these products.

The solvolysis products II, V, X and XI have been experimentally correlated with each other and with jervisine by reactions which cannot possibly affect the steric orientation of the hydroxyl group at C-23 and hence must all have the same configuration at that carbon atom, which moreover (since II is 11-ketoveratramine triacetate) must be the "natural" configuration pre-existing in veratramine and most likely also in jervine. Insofar as the mechanisms suggested above for the formation of the solvolysis products provide for retention of configuration at C-23, they are clearly in accord with the steric relationships just outlined. As regards C-17, an argument of sorts can be made for retention of configuration at this center also, on the following grounds: The "open" acetolysis product V and the quaternary salts X and XI must have the same configuration at this carbon atom, since these compounds are all convertible to jervisine mono- or triacetate under conditions precluding inversion (*i.e.*, with alkaline reagents, which could affect this center only if it carried a proton). Now V is transformed by the perchloric acid-containing acetolysis mixture in excellent yield to the perchlorate XI[1] under exactly the same conditions (room temperature, 2.5 hours) as is diacetyljervine in the reaction I → XI. Obviously, V → XI proceeds without inversion, and one could then well argue that this should be true also of I → XI, in which case all the solvolysis products as well as jervisine would have the "natural" configuration at C-17 present in jervine itself. The point we wish to make here is that even if retention in I → XI (and hence also I → VII → V, and N-acetyljervine → X), were strictly proved, this would not necessarily mean that these reactions (b, d and a above) are non-concerted, since retention may occur also in a SN2 reaction if the 13,17a-double bond should exert a neighboring group effect.

For the rearrangement of the quaternary salts X and XI to the jervisine derivatives VIII and IX, respectively, we suggest the sequence



which in view of the extraordinary facility with which the reaction proceeds is probably concerted.[15] The driving force may be supplied by the instability of the intermediary vinylidene amine Xa, for the formation of which we lean on the evidence of Leonard, *et al.*,[5,6] showing that quaternary Schiff base salts on alkalinization lose a proton from the β-carbon atom and thus pass into the tertiary vinylamine. The less facile rearrangement of the triacetate V, which requires strong alkali, is probably initiated by the hydrolytic cleavage of the bond between the nitrogen and the acetyl group, which then, for reason of steric proximity, accepts the electron pair of the ionized 17-hydroxyl group.

Jacobs and Pelletier,[16] and subsequently Barton, *et al.*,[17] have assigned to the tertiary veratrum alkamines cevine, germine and protoverine a hexacyclic ring system which differs from that of jervisine only in that the nitrogen atom is linked to C-18 instead of C-17a. It is obvious that these alkamines should be much stronger bases than jervisine, in which the nitrogen atom is subject to the shielding effect of the angular C-18 methyl group. We have determined the basic strength of germine and of

(15) This would presuppose that the nitrogen atom in X and XI is already in close proximity to C-17a. Scale models constructed on the premise that the side chain at C-17 and the 21-methyl group are oriented as in normal steroids shows that this condition is fairly well met in a number of stereoisomeric forms. Essential requirements are that C-20 is equatorial in respect to ring D (which for better visualization of the approach of the nitrogen atom to C-17a was made saturated, with the 18-methyl group β and axial), and that the hydrogen at C-22 is axial in respect to the piperidine ring F; the configuration given C-22 is relatively unimportant.

(16) W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.*, **18**, 765 (1953).

(17) D. M. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).

jervisine 17-monoacetate by titration with hydrochloric acid in aqueous dimethylformamide and found that while the pK_a of the former is in the normal range for tertiary bases (8.9), the buffering range of the jervisine derivative is in the pH 3 region. (The measurement could not be completed as the free base began to precipitate when pH 2.9 was reached.) This finding is in accord with the fact that the jervisine acetates VIII and IX do not form stable salts with hydrochloric acid and perchloric acid.[1]

Experimental

The melting points were taken in open Pyrex glass capillaries and are corrected for stem exposure. The rotation measurements were carried out in a 1 dm. semi-micro tube, with chloroform as the solvent, unless indicated otherwise. The ultraviolet spectra were measured in absolute ethanol in a Cary self-recording instrument model 11 M. The infrared spectra were determined on Nujol mulls in the Perkin–Elmer double beam self-recording spectrophotometer model 21. The characteristics of the infrared bands are expressed in the text as follows: (s) strong, (m) medium, (l) low, (br) broad, (sh) shoulder.

Treatment of N-Acetyljervine with Methanolic Hydrogen Chloride; Quaternary Chloride X.—N-Acetyljervine (4.88 g.) was dissolved in 125 ml. of absolute methanol which had been saturated at 0° with gaseous hydrogen chloride. The solution was allowed to stand at room temperature for one hour and was then taken to dryness *in vacuo*. The residue was distributed between chloroform (500 ml.) and water (300 ml.), and the aqueous phase extracted with an additional volume (75 cc.) of chloroform. The combined chloroform solutions were washed successively with 1 *N* hydrochloric acid, sodium carbonate solution and water, dried and evaporated to dryness. The residue (2.8 g.) on recrystallization from methanol–ethyl acetate gave 1.56 g. of N-acetylisojervine m.p. 203–204°.

The fine crystalline precipitate which had appeared in the aqueous phase was filtered off and washed with ice-cold water (1.65 g., m.p. 244–246°). An additional amount of the chloride (381 mg., m.p. 246–250°) was recovered from the filtrate after concentration to a small volume. The salt is only sparingly soluble in hot water or hot ethanol, but could be recrystallized by bringing it into solution in warm 50% aqueous ethanol and adding a few drops of hydrochloric acid. However, the recrystallized preparations invariably showed somewhat lower melting points and on analysis a slight deficit in chlorine. The crude product, m.p. 246°, was therefore used for the analysis after drying to constant weight in high vacuum at room temperature (weight loss 0.3%).

Anal. Calcd. for $C_{29}H_{42}O_4NCl$ (504.1): C, 69.09; H, 8.40; Cl, 7.03. Found: C, 69.32; H, 8.23; Cl, 7.21.

As with the normal N-acetyl derivatives of the jervine-veratramine series, only a fraction of the masked N-acetyl group in X was liberated as acetic acid in the Kuhn–Roth determination ($COCH_3$ calculated 8.54, found 1.3). Other properties: $[\alpha]^{24}D$ −53° (*c* 0.54 in 95% ethanol); λ_{max}^{alc} 243 mμ (17,100); 364 mμ (76); λ_{max}^{Nujol} 3.04(s), 3.10(s), 5.82(m), 6.10(s) μ.

A stream of ozone was passed through a solution of the chloride (365 mg.) in pure acetic acid (40 ml.). After addition of an equal volume of water the solution was slowly distilled in a stream of nitrogen into an ice-cooled receiver containing a few ml. of water. Three fractions, each representing about 2 ml. of distillate, were thus collected. To each fraction was added a 2 *N* sodium acetate solution (2 ml.) and 8% ethanolic dimedon (1 ml.). After warming on the steam-bath for 10 minutes the solutions were allowed to stand overnight. Each of them deposited a few milligrams of crystalline material (m.p. 175–177°) which was, however, also obtained under the same conditions from the reagent alone, and was identified as a trimeric self-condensation product, $C_{24}H_{32}O_4$, of dimedon.

Jervisine 17-Monoacetate (VIII) from Chloride X.—The chloride (50 mg.) was dissolved in methanol (5 ml.) with warming. The solution was brought to room temperature, and 2 *N* sodium carbonate solution (6 ml.) was added dropwise. The resulting crystalline precipitate was taken up, after addition of water (30 ml.), in ether. The residue of the water-washed and dried ether solution (50 mg.) was recrystallized twice from acetone–ether–pentane, yielding 32 mg. of platelets melting at 250–253°, $[\alpha]^{22}D$ −134° (*c* 0.861); the melting point of a mixture with jervisine 17-monoacetate (m.p. 243–253°, $[\alpha]D$ −133°) prepared by hydrolysis of the perchlorate XI[1] was not depressed. The infrared spectra of the two specimens were identical.

Reduction of Chloride X to Tertiary Base Hydrochloride XII.—The quaternary chloride X (1.64 g.) was suspended in warm ethanol (150 ml.) and brought into solution by the addition of water (12 ml.). After cooling the solution was admitted to a hydrogenation flask containing pre-reduced platinum oxide (960 mg.) suspended in ethanol (10 ml.). The uptake of hydrogen on shaking stopped after 70 minutes with the amount calculated for one molar equivalent (76 ml.) consumed. The crystalline precipitate which had formed was brought into solution by the addition of water (150 ml.) and slight warming. The solution was filtered from the catalyst and concentrated *in vacuo* to a small volume. The resulting crystalline precipitate was collected by centrifuging. After washing and drying it weighed 989 mg. and melted at 312–313°. In other runs occasionally products showing higher (up to 320°), but likewise sharp melting points were obtained; $[\alpha]^{26}D$ −69° (*c* 0.401 in 80% ethanol); λ_{max}^{alc} 249 mμ (13,000), 358 mμ (84); λ_{max}^{Nujol} 3.03(s), 5.85(s), 6.10(s) μ. The salt could be recrystallized in the manner described for X, but the preparation thus obtained showed a low chlorine content on analysis after drying to constant weight at 110°. On the other hand, the analyses of samples dried to constant weight at room temperature gave variable but always low figures for both carbon and chloride. Reasonably good figures indicating a dihydrate were obtained with a crude preparation dried in this manner (weight loss 4.7%).

Anal. Calcd. for $C_{29}H_{44}O_4NCl\cdot 2H_2O$ (542.1): C, 64.24; H, 8.92; Cl, 6.54. Found: C, 64.58; H, 8.81; Cl, 6.01.

For the preparation of the **free tertiary base XIII** the hydrochloride XII (316 mg.) was dissolved in methanol (50 ml.) and water (20 ml.) with warming, and aqueous sodium bicarbonate in excess was added to the solution slowly with stirring. The product, recovered by extraction with chloroform, was recrystallized from methanol, from which it formed square platelets (212 mg., m.p. 154–159°, unchanged by recrystallization), $[\alpha]^{25}D$ −80° (*c* 0.524); λ_{max}^{alc} 249 mμ (13,800); 357 mμ (84); λ_{max}^{Nujol} 2.86(m), 3.03 (m), 5.86(s), 6.14(m) μ. The analytical sample was dried to constant weight at 110° (0.2 mm.) (weight loss 5.9%).

Anal. Calcd. for $C_{29}H_{43}O_4N\cdot{}^1/_2H_2O$ (478.6): C, 72.76; H, 9.27. Found: C, 72.75; H, 9.30; equiv. weight (titrated with $HClO_4$ in acetic acid), 468.

Treatment of XII with methanolic 1 *N* potassium hydroxide either at room or reflux temperature (3 hours) yielded products of the same description, showing that the base is stable to strong alkali.

No acetaldehyde was liberated, and the hydrochloride was recovered unchanged, after the following treatments: refluxing of XII from 2 hours in a mixture of ethanol–water–concentrated hydrochloric acid 3:1:1; refluxing of XII for 4 hours with Brady reagent (1% 2,4-dinitrophenylhydrazine containing 1% hydrochloric acid). In another experiment the base XIII (48 mg.) was dissolved in 10% aqueous acetic acid (2.5 ml.), an equal volume of concentrated hydrochloric acid was added and the mixture was refluxed for 3 hours (under these conditions most of the hydrochloride stayed in solution). The material was recovered as the base by alkalization and chloroform extraction. Two recrystallizations from methanol yielded 24 mg. melting at 148–153°, undepressed by starting material; λ_{max}^{alc} 249 mμ (13,800), 358 mμ (95). Short heating of the base in a mixture of glacial acetic and concentrated hydrobromic acids lead to strongly pigmented, intractable products.

Acetylation of XIII with acetic anhydride and pyridine afforded the **3,23-diacetylated tertiary base XIV**. Recrystallization of the crude product (first from aqueous and then absolute methanol) gave clusters of needles, m.p. 201–202°, $[\alpha]^{25}D$ −77° (*c* 0.826); λ_{max}^{alc} 248 mμ (14,400), 355 mμ (94); λ_{max}^{Nujol} 5.76(s), 5.82(s), 5.86(s), 6.14(m), 7.95, 8.04 (doublet, s) μ.

Anal. Calcd. for $C_{33}H_{47}O_6N$ (553.7): C, 71.58; H, 8.56; 2 $COCH_3$, 15.55. Found: C, 71.15, 71.29; H, 8.52, 8.30; $COCH_3$, 16.3; equiv. weight ($HClO_4$ titr.), 552.

On hydrolysis with 5% methanolic potassium hydroxide (room temp., 18 hours) XIV reverted to the unacetylated base XIII, m.p. 151–157°, analysis correct for hemihydrate.

The hydrochloride XV of the diacetylated base was prepared by adding ethereal hydrogen chloride to an ether solution of XIV. The resulting precipitate after recrystallization from aqueous methanol melted at 247–252°; λ_{max}^{Nujol} 5.76(s), 5.88(s), 6.13(m), 8.09(s) μ.

Anal. Calcd. for $C_{33}H_{47}O_6N\cdot HCl\cdot{}^1/_2H_2O$ (599.2): C, 66.15; H, 8.24. Found: C, 66.21; H, 8.10.

In a previous experiment in which XIV was treated in ethanol with 5 *N* hydrochloric acid and isolation was effected by quickly boiling off the ethanol, the product was a dihydrate of the O-deacetylated hydrochloride XII (analysis, acetyl determination, absence of infrared band at 8 μ).

The perchlorate XVI of the diacetylated base XIV was obtained in an attempt to cleave the tetrahydrometoxazine ring of the unacetylated base XIII by treating this compound (42 mg.) with the perchloric-acid-containing acetolysis reagent (2 ml.) previously used on diacetyljervine.[1] The precipitate which formed after short standing was collected by centrifuging and washed with ethanol. Recrystallized from methanol it melted at 281–283°, $[\alpha]^{23}D$ −60° (*c* 0.443 in 80% ethanol); λ_{max}^{Nujol} 5.70(s), 5.78(s), 5.88(s), 6.10(m), 7.97(s) μ.

Anal. Calcd. for $C_{33}H_{47}O_6N\cdot HClO_4$ (654.2): C, 60.59; H, 7.40; 2 $COCH_3$, 13.16. Found: C, 60.72; H, 7.62; $COCH_3$, 12.2.

For the preparation of the salt from the quaternary perchlorate XI a solution (192 mg.) in 93% ethanol (30 ml.) was shaken in a hydrogen atmosphere with prereduced platinum oxide catalyst (123 mg.). The uptake continued at a slow rate after the consumption of one mole (15 minutes) and was interrupted at 1.3 moles. The twice recrystallized product melted at 265–267°, and in mixture with the specimen (m.p. 283°) obtained from XIII at 269–271°. Polymorphism rather than contamination with a perhydrogenated product is probably involved, as the analysis was correct (Found: C, 60.45; H, 7.34), and the absorption spectrum showed the normal characteristics (λ_{max}^{alc} 248 mμ (14,140), 358 mμ (86)). Furthermore, on decomposition with sodium bicarbonate the salt afforded in good yield the diacetylated base XIV.

Acetolysis of Tertiary Base XIII; 11-Ketoveratramine 3,23-Diacetate (III).—A solution of the diacetylated base XIV (201 mg.) in a freshly prepared mixture of acetic anhydride (7 ml.), acetic acid (3 ml.) and concentrated sulfuric acid (0.1 ml.) was allowed to stand at room temperature for 46 hours, and then poured onto ice. The mixture was brought to room temperature and, since it gave a faintly positive Schiff test, swept with a stream of nitrogen passing into dinitrophenylhydrazine reagent. However, no precipitate was formed in the latter. The somewhat turbid acidic solution was immersed in an ice-bath and made weakly alkaline by the gradual addition of sodium bicarbonate. The Schiff test was now strongly positive, and remained so after extraction of the acetolysis products with chloroform. The aqueous phase was placed into a distilling flask connected with a descending condenser and a receiver containing dinitrophenylhydrazine reagent (25 cc.), and subjected to slow distillation at atmospheric pressure till its volume was reduced by about one-third. The orange-colored precipitate in the receiver was collected (121 mg.) and recrystallized from ethanol containing 1% hydrochloric acid (for the removal of the co-precipitated dinitrophenylhydrazine base), and then repeatedly from pure ethanol. The resulting product (10 mg., m.p. 158–160°) when mixed with authentic acetaldehyde dinitrophenylhydrazone (m.p. 164–165°) melted at 159–161°. The infrared spectra of the two specimens were identical in every respect.

The yellow resinous residue of the water-washed, dried and evaporated chloroform extracts (152 mg.) was dissolved in benzene and chromatographed on a column of sulfuric-acid washed alumina. Continued washing with benzene eluted a crystalline product (21 mg.) identified as starting material. The following fractions, eluted with benzene–ether 9:1, were likewise crystalline. They were combined and recrystallized twice from ethyl acetate. 11-Ketoveratramine 3,23-diacetate was thus obtained as needles melting at 238–240.5°, $[\alpha]^{23}D$ −113° (*c* 0.586); λ_{max}^{alc} 250 mμ, (10,900), 303 mμ (1,920); λ_{max}^{Nujol} 3.04(l), 5.78(s), 5.82(s), 5.90(s), 6.27(l), 8.04(s) μ.

Anal. Calcd. for $C_{31}H_{41}O_5N$ (507.6): C, 73.34; H, 8.14. Found: C, 73.17; H, 8.00; equiv. weight ($HClO_4$ titration), 507.

Acetylation of III (15 mg.) with acetic anhydride in pyridine afforded the triacetate II,[1] m.p. 242–244°, undepressed by admixture of an authentic preparation; $[\alpha]^{24}D$ −27° (*c* 0.481). The infrared spectra of the two specimens were identical in every respect.

Sulfuric Acid Esters from Diacetyljervine.—The diacetate (2.0 g., 3.92 mmoles) was dissolved in a mixture consisting of acetic anhydride (28 ml.), acetic acid (12 ml.) and concentrated sulfuric acid (0.2 ml., 3.75 mmoles). After standing for 17 hours the solution was poured into water containing crushed ice, made slightly alkaline with sodium bicarbonate, and extracted with chloroform. The residue from the dried and evaporated extracts (1.81 g.) was dissolved in benzene and chromatographed on a column (25 × 110 mm.) of sulfuric acid-washed and reactivated alumina (50 g.). Elution was effected with 200-cc. portions of benzene, benzene–ether 9:1, 4:1 and 1:1, ether, ether–acetone 9:1, acetone, acetone–methanol 9:1 and 4:1 and methanol. Two crystalline products were obtained in small amounts (17 and 99 mg. after purification) from the benzene and benzene–ether 9:1 eluates, respectively. Since they were of doubtful purity and showed ultraviolet characteristics markedly different from those of II and V (single high maxima at 299 and 320 mμ), they were not further investigated. The bulk of the material (over 1 g.) remained absorbed on the column till acetone–methanol 9:1 and pure methanol were used for elution. The amounts released by these eluents were 222 and 851 mg., respectively. The acetone–methanol fractions on trituration with methanol yielded crystals, which were combined and recrystallized repeatedly from the same solvent. The pure product (30 mg., m.p. 208–209° dec., λ_{max}^{alc} 244 mμ (16,500); λ_{max}^{Nujol} 2.45 (l), 3.00(s), 5.76(sh., s), 5.81(s), 6.10(s), 8.07(br., s) μ) was found to contain sulfur, but not in the form of sulfate ion. The analyses were in agreement with the values required for the sulfuric acid ester VII.

Anal. Calcd. for $C_{33}H_{46}O_7N\cdot HSO_3$ (649.8): C, 61.00; H, 7.29; S, 4.93. Found: C, 60.62; H, 7.36; S, 5.02.

A solution of a sample (4.8 mg.) in a mixture of ethanol (1 ml.) and 1 *N* hydrochloric acid (1 ml.) was refluxed for 4 hours and after cooling treated with excess barium chloride. The barium sulfate formed weighed 1.60 mg. (calcd. 1.56 mg.).

To a solution of a sample (2.16 mg.) in ethanol (5 ml.) showing λ_{max} 243 mμ a small drop of 5% ethanolic potassium hydroxide was added; after one hour the spectrum showed λ_{max} 250 mμ (11,000) and a shoulder at 295 mμ (850).

Since the methanol eluates yielded no crystalline products, they were combined and lyophilized. This material, which showed λ_{max}^{alc} 248 mμ (5040), 319 mμ (4540), was found to contain sodium as well as sulfur in the amounts required by a sodium salt of VII.

Anal. Calcd. for $C_{33}H_{46}O_7N\cdot SO_3Na$ (671.8): S, 4.77; Na, 3.42. Found: S, 4.63; Na, 3.24.

The ultraviolet spectrum of an ethanolic solution remained essentially unaltered on addition of hydrochloric acid or potassium hydroxide solution.

A solution of the salt in a mixture consisting of equal volumes of ethanol, water and 20% hydrochloric acid gave no precipitate with barium chloride on prolonged standing, but yielded the theoretical amount of barium sulfate after refluxing for several hours. In a preparative experiment with 314 mg. of the salt in 60 ml. of the above mixture the solution was boiled for one hour, brought after cooling to *p*H 8 with sodium carbonate, and extracted with chloroform. The re-acidified aqueous phase yielded with barium chloride 86 mg. of barium sulfate (calcd. 109 mg.). The residue of the dried and evaporated chloroform extract (248 mg.) was reacetylated with acetic anhydride and pyridine, and the resulting product (214 mg.) was chromatographed on sulfuric acid-washed alumina in the usual manner. The benzene–ether 9:1 eluates (100 mg.) contained a crystalline product which after repeated recrystallization from aqueous ethanol melted at 226–230°, $[\alpha]^{24}D$ +1° (*c* 0.830). It was free of chlorine, and from its analysis and spectral characteristics (λ_{max}^{alc} 253 mμ (14,800)), 350 mμ (158); λ_{max}^{Nujol} 5.76(s), 5.90(s), 6.14(s), 8.06(s) μ) appeared to be a stereo-

isomer of diacetyljervine (m.p. 173–175°, $[\alpha]_D$ −112°). The analytical sample was dried at 137° (0.1 mm.) for 3 hours.

Anal. Calcd. for $C_{31}H_{43}O_5N$ (509.7): C, 73.05; H, 8.50. Found: C, 73.22, 72.75; H, 8.21, 8.19.

The rotation and analytical findings were confirmed on a specimen obtained in a repetition of the experiment.

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[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY, UNIVERSITY OF CHICAGO, AND THE ARGONNE NATIONAL LABORATORY]

The Synthesis of Poly-α-amino Acids in Anhydrous Hydrogen Fluoride[1]

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Solutions in anhydrous hydrogen fluoride of N-carboxyanhydrides of α-amino acids (NCA's) or of α-aminoacyl chloride hydrochlorides react to yield solutions of amino acid polymers. The reaction is complete within several hours at room temperature and has been made to yield polymers with average chain lengths of 25 to 30 units. Polymers have been prepared from the NCA's of DL- and L-leucine, L-alanine and DL-phenylalanine. Racemization occurs to the extent of about 40%; diketopiperazines are significant by-products. Sarcosine NCA yields chiefly N,N′-dimethyldiketopiperazine. It also has been possible to initiate polymerization in a sulfur dioxide solution or in the bulk NCA by addition of traces of hydrogen fluoride.

A recent report of the use of anhydrous hydrogen fluoride as a powerful solvent for proteins and polypeptides[2] has prompted an exploration into the possibility of its use as a solvent for the preparation of polypeptides. A growing peptide chain would not be likely to precipitate from hydrogen fluoride solution; thus one possible cause of chain termination would be eliminated, especially in the case of the simple bifunctional amino acids whose polymers are generally highly insoluble in other solvents. In the course of such a study, it has been found that N-carboxyanhydrides (NCA's) of amino acids, when dissolved in hydrogen fluoride, lose carbon dioxide and are converted to peptides.

N-Carboxyanhydrides of amino acids have been widely used for preparation of polymers and copolymers of amino acids.[3] Commonly, a solution of the anhydride is treated with an amine, alkoxide or hydroxide to initiate polymerization. Recently, polymerization has been induced by use of certain salts or tertiary amines in polar solvents. The mechanism of these polymerizations has been extensively investigated.[4a,b] A degree of control over chain length may be achieved by varying the concentration of the initiator used. On the other hand, the action of hydrogen chloride in ethanol on the N-carboxyanhydrides has been reported to lead to hydrochlorides of amino acid ethyl esters.[5] The present work indicates that L-leucine NCA may be converted to L-leucyl chloride hydrochloride by action of hydrogen chloride in toluene, although it may be recovered unchanged from anhydrous trifluoroacetic acid after several days storage at room temperature. In view of these facts, the polymerization which has been found to occur in the presence of hydrogen fluoride is perhaps unexpected.

The NCA's used in this study were those derived from L-alanine, DL- and L-leucine, DL-phenylalanine and sarcosine, although the bulk of the studies were carried out with leucine derivatives. These were placed in Fluorothene or nickel tubes attached to a suitable vacuum line. The reaction tubes were held at liquid nitrogen temperature as hydrogen fluoride was distilled in, then closed off from the line and brought to a suitable temperature. At room temperature or above there occurred appreciable gas evolution, which continued for about an hour. After removal of solvent by distillation under vacuum the infrared spectra of the products were measured without further manipulation. These spectra indicated that the products were peptide in nature, of the α-, folded, configuration.[6] In those cases in which thorough removal of solvent was effected, the loss of weight corresponded to one equivalent of carbon dioxide. The raw polymer was washed with water or water–dioxane to hydrolyze any remaining reactive functional groups and at the same time to separate the material into soluble and insoluble portions. In the case of the DL-leucine polymers the soluble product, about 15% of the total, proved to be chiefly 3,6-diisobutyl-2,5-diketopiperazine. Varying amounts of corresponding diketopiperazines were noted in the soluble fractions from other runs. In all cases the insoluble product was polypeptide and afforded only the corresponding amino acid on hydrolysis. No hydantoin-3-acetic acid derivatives were found among the reaction products nor were cyclic peptides other than diketopiperazines isolated. The optically active N-carboxyanhydrides used led to products which were as much as 40% racemized, although the amino acids themselves were unchanged by storage in hydrogen fluoride. Sarcosine N-carboxyanhydride, when treated with hydrogen fluoride, afforded a 60% yield of sarcosine anhydride, 1,4-dimethyldiketopiperazine, in contrast to a maximum of 20% diketopiperazine found

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

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