whey, the proteins of whey were extracted with alcohol by the method used for preparing γ-casein. A small amount of whey protein was found to be soluble in 50% alcohol at room temperature. The electrophoretic mobility of the alcohol-soluble whey protein was greater, however, than that of γ-casein.

A further attempt was made to demonstrate the presence of γ-casein in whey by heat coagulating the proteins of whey at *p*H 4.7. It might be expected that γ-casein would be present in the filtrate after heat coagulation, since γ-casein is not coagulable by heat. Electrophoretic analysis of the protein of whey not coagulable by heat, however, did not reveal γ-casein. Osborne and Wakeman[4] were unable to demonstrate the presence of their alcohol-soluble casein in whey by extraction of the whey proteins with alcohol, but they obtained anaphylactic reactions with the protein not coagulable by heat, which they interpreted as indicating the presence of their alcohol-soluble casein. The apparent complete removal of γ-casein in the precipitation of the casein fraction from skim milk at *p*H 4.7 may be in part explained by the effect of the salt present in decreasing its solubility, as was demonstrated by Linderstrøm-Lang and Kodama[6] for unfractionated casein in solution acid to the isoelectric point and in part by its complex formation with α- and β-casein.

The sulfur and phosphorus contents of γ-casein (Table I) are approximately the same as those reported by Osborne and Wakeman for their alcohol-soluble casein. γ-Casein also resembles the alcohol-soluble casein in that its aqueous solutions become opaque when warmed. β-Casein solutions, however, behave in a similar manner. The sulfur contents of α- and β-casein are consistent with the estimates of the sulfur-containing amino acids reported by Gordon, *et al.*[7]

The optical rotations of the three casein fractions are consistent with the rotation $(\alpha)^{25}D$ − 105 of unfractionated casein, based on the composition of unfractionated casein determined by electrophoretic analysis—approximately 75% α-casein, 22% β-casein and 3% γ-casein.

Acknowledgment.—We are indebted to C. L. Ogg of this Laboratory for the sulfur analyses.

Summary

A method is described for separating γ-casein from α- and β-casein that involves alcohol–water fractionation and isoelectric precipitations. In composition and properties, γ-casein is similar to the alcohol-soluble casein described by Osborne and Wakeman.

(7) Gordon, Semmett, Cable and Morris, THIS JOURNAL, 71, 3293 (1949).

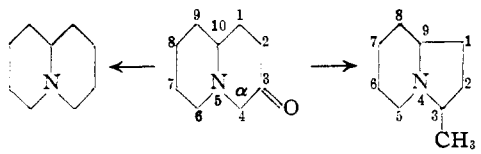
PHILADELPHIA 18, PA. RECEIVED APRIL 12, 1950

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Rearrangement of α-Aminoketones during Clemmensen Reduction. VI. The Rearrangement of 3-Ketoquinolizidine

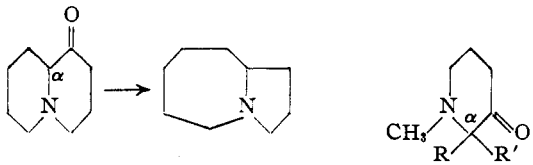
BY NELSON J. LEONARD AND SEEMON H. PINES

In the midst of mounting evidence for rearrangement generally accompanying the Clemmensen reduction of cyclic α-aminoketones,[1] the "normal" reduction of 3-ketoquinolizidine (I)[2] to quinolizidine (II),[3] reported by Clemo, Morgan and Raper,[4] stands out in striking nonconformity. This is especially true since it has



II I III

been established[5] that the isomeric α-aminoketone, 1-ketoquinolizidine (IV), undergoes rearrangement during Clemmensen reduction conditions to give 1-azabicyclo[5.3.0]decane (V), and since it has also been shown[6] that rearrangement *cum* reduction occurs in the analogous 1-methyl-3-piperidone series (VI) no matter whether the α-carbon is unsubstituted (*cf.* I), monoalkyl-substituted (*cf.* IV), or dialkyl-substituted. We have therefore chosen to repeat the Clemmensen reduction of 3-ketoquinolizidine (I)[2,4] in the belief that the main product should not be quinolizidine but, instead, a mixture of the racemates represented by III, and resulting from ketonic ring contraction.[7] The use of chromatographic adsorption and the determination of infrared spectra



IV V VI

(1) For leading references, see (a) Leonard and Wildman, THIS JOURNAL, 71, 3100 (1949); (b) Clemo, Raper and Vipond, *J. Chem. Soc.*, 2095 (1949).

(2) Alternatively named 3-ketooctahydropyridocoline.

(3) Alternatively named norlupinane.

(4) Clemo, Morgan and Raper, *ibid.*, 1743 (1935).

(5) Prelog and Seiwerth, *Ber.*, 72, 1638 (1939).

(6) Leonard and Barthel, THIS JOURNAL, 72, 3632 (1950).

(7) Leonard and Wildman, *ibid.*, 71, 3089 (1949).

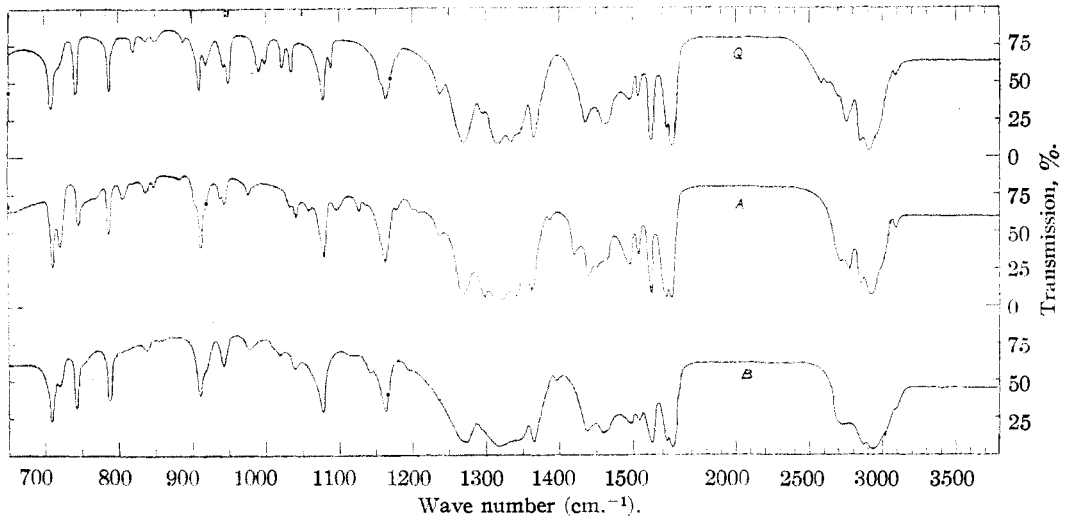
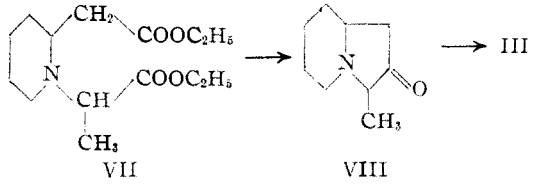


Fig. 1.—Infrared absorption of picrates.

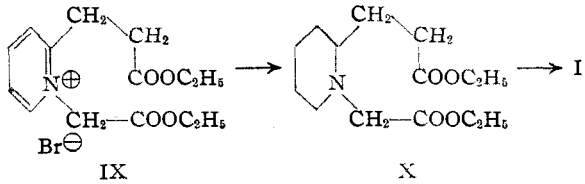
have been especially useful tools in establishing the conversion of I to III as a fact.

For comparison purposes, authentic 3-methyloctahydropyrrocoline (III) was made by one of the methods[4,8] employed by Clemo and his co-workers, namely, the Dieckmann ring closure of diethyl piperidyl-1-α-propionate-2-acetate (VII), followed by Wolff–Kishner reduction of the 2-keto-3-methyloctahydropyrrocoline (VIII) obtained on hydrolysis of the condensation product. It was possible to isolate both racemates of



III by means of chromatographic absorption of the Wolff–Kishner product on activated alumina, and these were characterized by the formation of derivatives: A, picrate, m. p. 195–197°; picrolonate, m. p. 216–218°, and B, picrate, m. p. 215–216°; picrolonate, m. p. 166–168°.

For the synthesis of 3-ketoquinolizidine (I), the method of Clemo, Morgan and Raper (IX → X → I)[4] was followed closely, with careful characterization at each stage (IX, X) so as to ensure the identity of the final α-aminoketone (I).



The base resulting from the Clemmensen reduction of 3-ketoquinolizidine (I) was distilled, and the distillate was subjected to chromatography on activated alumina. The two major products thus isolated were characterized by the formation of derivatives: A, picrate, m. p. 195–197°; picrolonate, m. p. 216–218°, and B, picrate, m. p. 215–216°; picrolonate, m. p. 166–168°. These were identified as the "A" and "B" racemates of 3-methyloctahydropyrrocoline (III) by comparison of melting points and infrared spectra. The infrared spectrum of the picrate of "A" from Clemmensen reduction is identical with that of the picrate of "A"-3-methyloctahydropyrrocoline (Fig. 1), and the same is true for the infrared spectra of the "B" picrates. By contrast, the infrared spectrum of quinolizidine picrate (Q, Fig. 1) is markedly different from both of these, whereas the melting point (197.5–198.5°)[1a] of quinolizidine picrate lies in the same range as that of the picrate of "A" and is depressed only slightly on mixing with the latter.[9]

Our findings indicate that the two racemates of 3-methyloctahydropyrrocoline (III) constitute at least 95% of the $C_9H_{17}N$ product obtained in the Clemmensen reduction of 3-ketoquinolizidine (I). Accordingly, this α-aminoketone (I) has now been shown to behave similarly to other cyclic α-aminoketones[1] in undergoing ketonic ring contraction under Clemmensen reduction conditions.

Experimental[10]

3-Ketoquinolizidine

This compound[2] was prepared essentially by the method of Clemo, Morgan and Raper.[4] Since the identity of this α-aminoketone was crucial to the findings on Clemmensen

(8) Clemo, Metcalf and Raper, *J. Chem. Soc.*, 1429 (1936).

(9) For their statement that I underwent reduction without rearrangement, Clemo, Morgan and Raper[4] relied upon the observation that the base they isolated from the Clemmensen reduction of I gave a picrate, m. p. 194°, and picrolonate, m. p. 245° ("Several recrystallizations of the derivatives are necessary before the melting points attain constant values"), which did not depress the corresponding derivatives of quinolizidine.

(10) All melting points are corrected. Microanalyses were performed by Miss Emily Davis, Miss Rachel Kopel and Mr. Maurice Dare.

reduction, each intermediate was carefully characterized by physical properties and analysis, as indicated below.

Diethyl Pyridinium-1-acetate-2-β-propionate Bromide.—The quaternary salt (IX) was formed in 73% yield by refluxing equimolar quantities of ethyl bromoacetate and ethyl β-(2-pyridyl)-propionate in anhydrous acetone. After recrystallization from acetone containing 8% ethanol, the colorless needles melted with decomposition at 158–159° (reported, 159°[4]).

Anal. Calcd. for $C_{14}H_{20}BrNO_4$: C, 48.56; H, 5.82; N, 4.05. Found: C, 48.33; H, 6.04; N, 4.29.

Diethyl Piperidyl-1-acetate-2-β-propionate.—Hydrogenation of IX over platinum oxide catalyst in aqueous acetic acid gave X in 82% yield; b. p. 112–113° (0.35 mm.) (reported, 138–140° (1 mm.)[4]); $n^{20}D$ 1.4645.

Anal. Calcd. for $C_{14}H_{25}NO_4$: C, 61.96; H, 9.29; N, 5.16. Found: C, 62.22; H, 9.48; N, 5.18.

Dieckmann Ring Closure of Diethyl Piperidyl-1-acetate-2-β-propionate. 3-Ketoquinolizidine.—Standard treatment with potassium sand in refluxing toluene, followed by hydrolysis and decarboxylation, gave the aminoketone (I) in 40% yield; b. p. 62–63° (0.65 mm.) (reported, 74–76° (1 mm.)[4]); $n^{20}D$ 1.4926. The clear liquid darkened rapidly on exposure to air.

Anal. Calcd. for $C_9H_{15}NO$: C, 70.55; H, 9.87. Found: C, 70.82; H, 10.39.

The **picrate** was prepared in ether and recrystallized from acetone at low temperature; m. p. 180–182°, with decomposition (reported, 185°[4]).

Anal. Calcd. for $C_{15}H_{18}N_4O_8$: C, 47.12; H, 4.75; N, 14.65. Found: C, 47.17; H, 4.85; N, 14.45.

Clemmensen Reduction of 3-Ketoquinolizidine.—The reduction was carried out in the usual fashion.[1] The reaction solution was concentrated *in vacuo*, made distinctly alkaline with potassium hydroxide, and the mixture was steam-distilled. The distillate was saturated with potassium carbonate, extracted with ether, and the ethereal solution was dried over anydrous magnesium sulfate. The ether was removed through an eight-inch Fenske column, and the amine which distilled in the range 53–64° (13 mm.) was collected. A solution of 0.98 g. of distillate in 100 ml. of petroleum ether (b. p. 37–53°) was passed through a 24-cm. column of activated alumina. Percolation of the column with petroleum ether followed by benzene–petroleum ether and benzene brought out two distinctly different amines, isolated as their picrates. Isomer "A" was obtained from the petroleum ether percolates and isolated as the **picrate**, compact yellow prisms, m. p. 195–197° (dec.) after one recrystallization from methanol. Isomer "B" was obtained from the latter two percolates and formed a **picrate**, yellow needles, m. p. 215–216° (dec.) after one recrystallization from methanol.

Of the total picrate recovered (75% of the theoretical quantity) 60% was "A" picrate and 35% was "B" picrate, and the fraction collected between "A" and "B" formed a picrate which was obviously a mixture, m. p. 204–209° (dec.). The ratio of "A" to "B" was reproducible in other identical experiments. No amine was obtained on percolation of the alumina column by solvents: ether through ethanol.

Anal. Calcd. for $C_{15}H_{20}N_4O_7$: C, 48.91; H, 5.47; N, 15.22. Found: "A" picrate: C, 49.01; H, 5.38; N, 15.10; "B" picrate: C, 48.94; H, 5.68; N, 15.01.

A small portion of each picrate was decomposed, and each base was converted to a picrolonate: "A" picrolonate, yellow plates, m. p. 216–218° (dec.); "B" picrolonate, orange elongated prisms, m. p. 166–168°. Both were recrystallized from ethanol.

Anal. Calcd. for $C_{19}H_{25}N_5O_8$: C, 56.56; H, 6.25; N, 17.36. Found: "A" picrolonate: C, 56.72; H, 6.45; N, 17.47; "B" picrolonate: C, 56.53; H, 6.42; N, 17.31.

3-Methyloctahydropyrrocoline

This compound had been prepared previously[4,8] but the *two* racemates had not been obtained. Again, in the synthesis of 3-methyloctahydropyrrocoline it was considered necessary to characterize each intermediate carefully so as to be sure of authentic product.

Diethyl Piperidyl-1-α-propionate-2-acetate.—The diester (VII) was made by the method of Clemo, Morgan and Raper,[4] by allowing ethyl α-bromopropionate to react with ethyl 2-piperidylacetate in the presence of anhydrous potassium carbonate: b. p. 103° (0.35 mm.) (reported, 135–140° (1 mm.)[4]), $n^{20}D$ 1.4634, yield, 73%.

Anal. Calcd. for $C_{14}H_{25}NO_4$: C, 61.96; H, 9.29; N, 5.16. Found: C, 61.83; H, 9.32; N, 5.42.

Dieckmann Ring Closure of Diethyl Piperidyl-1-α-propionate-2-acetate. 2-Keto-3-methyloctahydropyrrocoline.—Standard treatment with potassium sand in refluxing xylene, followed by hydrolysis and decarboxylation, gave the aminoketone (VIII) in 71% yield[11]: b. p. 75–76° (3.9 mm.) (reported, 67–69° (1 mm.)[4]); $n^{20}D$ 1.4782.

Anal. Calcd. for $C_9H_{15}NO$: N, 9.14. Found: N, 9.08.

The **picrate**, formed in ether and recrystallized from ethanol, was obtained as yellow prisms, m. p. 158–160° (dec.) (reported, 162°).[4]

Anal. Calcd. for $C_{15}H_{18}N_4O_8$: C, 47.12; H, 4.75; N, 14.65. Found: C, 47.10; H, 4.87; N, 14.62.

The **picrolonate**, formed in ethanol and recrystallized from methanol, was obtained as orange prisms, m. p. 187–189° (dec.).

Anal. Calcd. for $C_{19}H_{23}N_5O_8$: C, 54.67; H, 5.55; N, 16.78. Found: C, 54.94; H, 5.70; N, 16.91.

3-Methyloctahydropyrrocoline.—The Wolff–Kishner reduction of 2-keto-3-methyloctahydropyrrocoline was carried out in the usual manner,[12] and the base boiling over the range 42–46° (4.5 mm.) (48% yield) was subjected to chromatographic separation on an alumina column as with the product of Clemmensen reduction of 3-ketoquinolizidine. The two racemates were isolated as **picrates**: "A" picrate, compact yellow prisms from methanol, m. p. 195–197° (dec.); "B" picrate, yellow needles from methanol, m. p. 215–216° (dec.) (one picrate, m. p. 197°, and one picrolonate, m. p. 208° (*cf.* above) reported by Clemo and co-workers).[4,8] The "A" and "B" derivatives were identical with those obtained from the Clemmensen reduction of 3-ketoquinolizidine. The isomer "A" was present to the extent of about 90% in the base which was chromatographed.

Infrared Spectra.[13]—The picrates obtained from the product of the Clemmensen reduction of 3-ketoquinolizidine, isomers "A" and "B," were found to have infrared spectra identical (A, B in Fig. 1) with those of the authentic picrates of isomers "A" and "B," respectively, of 3-methyloctahydropyrrocoline obtained from the Wolff–Kishner reduction of 2-keto-3-methyloctahydropyrrocoline. The spectrum of an authentic sample of quinolizidine picrate (Q in Fig. 1) was different enough from the spectra of both "A" and "B" picrates that a pure specimen could have been detected if it had been isolated from the Clemmensen-reduction chromatography. The spectra were obtained from Nujol suspensions of the respective picrates.

The infrared absorption spectrum of a solution containing about 4% quinolizidine and 96% of the two racemates of 3-methyloctahydropyrrocoline was compared with the spectra of pure quinolizidine and of 3-methyloctahydropyrrocoline (both racemates). This comparison indicated that such an analysis of the Clemmensen reduction product for small amounts of quinolizidine would be inconclusive.

(11) The improvement in yield is probably due to the use of only a slight excess of potassium (0.05–0.1% atom) as contrasted to previous use of one atom excess.

(12) Leonard and Barthel, THIS JOURNAL, 71, 3098 (1949).

(13) The authors are indebted to Miss Elizabeth M. Petersen for determination of the infrared absorption spectra.

Summary

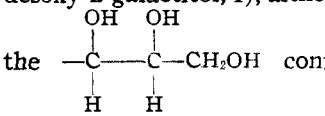
Both racemates of 3-methyloctahydropyrrocoline have been obtained for the first time. It has been shown that these compounds, rather than quinolizidine, are produced predominantly in the Clemmensen reduction of 3-ketoquinolizidine.

URBANA, ILLINOIS RECEIVED MAY 19, 1950

[CONTRIBUTION FROM THE EXPERIMENTAL BIOLOGY AND MEDICINE INSTITUTE, NATIONAL INSTITUTES OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

L-Fuco-4-ketose, a New Sugar Produced by the Action of *Acetobacter suboxydans* on L-Fucitol[1]

BY NELSON K. RICHTMYER, LAURA C. STEWART AND C. S. HUDSON

Earlier work from this laboratory by Hann, Tilden and Hudson[1a] has shown that L-fucitol (6-desoxy-L-galactitol, I), although it does not possess the  configuration that they found to be favorable for the ready oxidation of polyhydric alcohols to 2-ketoses by the action of *Acetobacter suboxydans*, was indeed attacked by that organism to a very appreciable extent. The solution resulting from their oxidation of L-fucitol showed a specific rotation of $-7°$. Recently Bollenback and Underkofler[2] have confirmed this biochemical oxidation, and by a reinoculation technique have obtained a 68% oxidation (calculated as glucose) of L-fucitol after twenty days.

The object of our research was to identify the product of this biochemical oxidation of L-fucitol. Under the conditions used, the reaction appeared to be complete by the fifteenth day with a 62% conversion to reducing sugar (calculated as glucose). Deproteinization followed by deionization yielded a solution of the reducing material whose $[\alpha]^{20}D$ value was estimated to be $-5°$. Upon concentration of this solution 13.7 g. of the original 38.7 g. of L-fucitol could be recovered in crystalline form. The remainder of the material was a sirup (20.6 g.) which did not show any inclination to crystallize. With phenylhydrazine it yielded neither L-fucose phenylhydrazone nor phenylosazone, from which we concluded that the principal component was neither L-fucose nor L-fuculose (II); furthermore the rotation of L-fuculose[3] is positive, whereas our product was levorotatory. Hence the new and unknown sugar must have been formed through oxidation of a secondary hydroxyl group on carbon 3, 4 or 5.

To distinguish among these three possibilities the sirup was hydrogenated in the presence of Raney nickel. Each of the three possible carbonyl compounds would be expected to yield L-fucitol and in addition an isomeric alcohol whose identification would then enable us to recognize the parent ketose. Thus if the CHOH group on carbon 3 were oxidized with the formation of compound III, the latter on subsequent reduction should furnish L-fucitol (I) and 6-desoxy-L-gulitol (VI). This last substance, known from the work of Müller and Reichstein[4] and of Bollenback and Underkofler,[2] melts at 133–134° and crystallizes readily. However, after removal of the L-fucitol the inoculation of our residual sirup under favorable conditions with an authentic sample of 6-desoxy-L-gulitol failed to induce crystallization. Formula III was thereby eliminated from further consideration.

Formula V was next discarded when our sirup failed to crystallize when inoculated with 6-desoxy-D-altritol (VIII), a new substance which we prepared especially for this purpose from the methyl 2,3,4-tribenzoyl-6-desoxy-α-D-altroside described in a recent paper from this laboratory.[5] Confirmatory evidence that the second alcohol was not 6-desoxy-D-altritol was found in the latter's levorotation, $[\alpha]^{20}D$ $-9.4°$ in water, whereas the unknown sirupy alcohol showed dextrorotation, $[\alpha]^{20}D$ $+5.4°$.

That left formula IV as the remaining possibility for the new sugar. Upon hydrogenation of a ketose of this structure there should be obtained as the second alcohol 6-desoxy-L-glucitol (VII), usually called L-epirhamnitol. This substance had been prepared as a sirup by Votoček and Mikšič,[6] and its rotation reported as $[\alpha]^{20}D$ $+9.18°$ in water. Although those authors had prepared a crystalline dibenzylidene derivative, we chose to complete the identification of our presumed L-epirhamnitol (VII) by condensing it with formaldehyde in the presence of concentrated hydrochloric acid. Ness, Hann and Hudson[7] had proved that the product obtained in this way from the enantiomorphous alcohol was 1,3:2,4-dimethyl-

(1) Presented in part before the Division of Sugar Chemistry and Technology at the Detroit meeting of the American Chemical Society, April 18, 1950.

(1a) R. M. Hann, E. B. Tilden and C. S. Hudson, THIS JOURNAL, **60**, 1201 (1938).

(2) G. N. Bollenback and L. A. Underkofler, *ibid.*, **72**, 741 (1950).

(3) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, **20**, 1529 (1937); **21**, 913 (1938).

(4) H. Müller and T. Reichstein, *ibid.*, **21**, 251 (1938).

(5) D. A. Rosenfeld, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **70**, 2201 (1948).

(6) E. Votoček and J. Mikšič, *Bull. soc. chim. France*, [4] **43**, 220 (1928).

(7) A. T. Ness, R. M. Hann and C. S. Hudson, THIS JOURNAL, **66**, 1235 (1944).