(93% based on converted starting material) of III, b.p. 87–90° (1.5 mm.).[6]

When 1.23 equiv. of peroxytrifluoracetic acid and BF_3 was used, an 88% yield of III was obtained and only 4% of unchanged hexamethylbenzene remained. When BF_3 was omitted, the conversion was only 70%, although the yield of III was 86%.

Acknowledgment.—We are grateful to the National Science Foundation (GP-71) for financial support of this work. A. J. W. also wishes to thank the United States Educational Commission in the United Kingdom for the award of a Fulbright Travel Grant.

(6) New compounds III, IV, V, and IX gave correct microanalyses.

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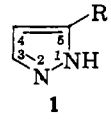
RECEIVED FEBRUARY 15, 1964

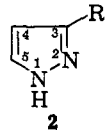
Heterocyclic Studies. XI.[1] The Tautomeric Structure of 3(5)-Methyl-4-phenylpyrazole

Sir:

It has long been recognized that unsymmetrical pyrazoles can exist in two tautomeric forms, **1** and **2**. von Auwers[2] concluded from molecular refraction exaltations that the 3-phenyl tautomers (**2**, R = C_6H_5) predominated in 3(5)-phenylpyrazole and methyl 3(5)-phenylpyrazole-5(3)-carboxylate. This method was applicable only to 3(5)-arylpyrazoles, and little further work on the question of pyrazole tautomerism has been done.[3] The data presented below indicate that the tautomeric structure of 3(5)-substituted pyrazoles can be determined from n.m.r. measurements by the method used by Moore and Whittaker to establish the structure of tetrazole.[4]

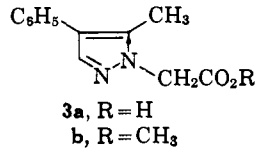


1

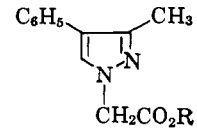


2

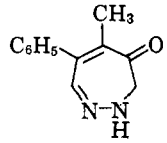
A major problem in approaching the question of pyrazole tautomerism is the difficulty of obtaining N-alkyl pyrazole isomers of rigorously known structure.[5] We have now prepared 3- and 5-methyl-4-phenylpyrazole-1-acetic acids by conventional syntheses from 1-ethoxymethylene-1-phenylacetone and ethyl hydrazinoacetate, and also by alkylation of 3(5)-methyl-4-phenylpyrazole[7] with methyl bromoacetate: 3-methyl isomer (**4**), m.p. 212°, λ_{max}^{EtOH} 245 mμ, methyl ester, m.p. 60°; 5-methyl isomer (**3**), m.p. 212°, λ_{max}^{EtOH} 244 mμ, methyl ester, m.p. 91°.[8] A firm basis for the structural assignments is provided by the formation of the latter compound (5-methyl isomer) in 50% yield by alkaline hydrogen peroxide oxidation of the diazepinone **5**.[9]



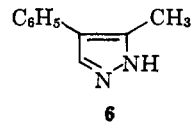
3a, R = H
b, R = CH_3



4a, R = H
b, R = CH_3



5



6

The proton n.m.r. spectra of the methyl esters **3b** and **4b** and of the unsubstituted pyrazole **6** were obtained in $CDCl_3$ solution (tetramethylsilane internal standard) with a Varian A-60 instrument. The spectra of **3b** and **4b** were obtained at solution concentrations of 2 and 8% (w./w.) and that of the less soluble pyrazole **6** at 1 and 4% concentrations. In contrast to the marked concentration dependence of the 2-proton peak in indole n.m.r. spectra,[10] the positions of all peaks, including that of the 3(5)-proton, in these pyrazole spectra were constant over a fourfold change in concentration within the reproducibility (±1.0 c.p.s.) of the measurements.

The spectra of **3b** and **4b** contained single peaks corresponding to 3(5)-CH_3, $-OCH_3$, $-NCH_2CO$, C_6H_5, and 5(3)-H in the correct intensities; that of **6** had sharp peaks for 3(5)-CH_3, C_6H_5, and 5(3)-H. The –NH signal was a broadened peak at δ 11.50 p.p.m.; there was no multiplicity in any of the peaks. The peak positions for the 3- or 5-proton, 4-aryl protons, and 5- or 3-methyl protons are shown in Table I.

TABLE I

Compound	Chemical shift, δ[a]			Δ	
	3- or 5-H	C_6H_5	5- or 3-CH_3	3(5)-H–C_6H_5	3(5)-H–CH_3
3b	7.62	7.33	2.30	0.29	5.32
4b	7.49	7.37	2.40	0.12	5.09
6	7.70	7.34	2.46	0.36	5.24

[a] Average of three values, in p.p.m.

The differences in chemical shifts and spacing (Δ) of the 3- or 5-proton peaks and the phenyl and methyl peaks of **3b** and **4b** are evidently due, as in the case of 1H- and 2H-tetrazole isomers,[4] to the difference in double bond positions in the two isomers. Although the difference in chemical shifts of the ring protons (0.13 p.p.m.) in **3b** and **4b** is considerably less than that (0.6–0.8 p.p.m.) in the tetrazole case, and would be too small to permit assignment of the tautomeric structure of **6**, comparison of the spacings (Δ) of this signal and those of the phenyl and methyl groups in **3b** and **4b** permits a tentative conclusion that the predominant tautomer of **6** possesses the same double bond system as **3b**. It is hoped to test the validity of this conclusion when ad-

(1) Paper X: J. A. Moore and L. J. Pandya, *J. Org. Chem.*, **29**, 336 (1964).

(2) K. von Auwers, *Ann.*, **508**, 51 (1933).

(3) A recent review has been presented by A. R. Katritzky and J. M. Lagowski, "Advances in Heterocyclic Chemistry," Vol. 2, Academic Press, New York, N. Y., 1963, p. 31.

(4) D. W. Moore and A. G. Whittaker, *J. Am. Chem. Soc.*, **82**, 5007 (1960).

(5) Structures were assigned to a number of isomeric pairs of N-alkyl pyrazoles, *e.g.*, 1,3- and 1,5-dimethylpyrazole (K. von Auwers and H. Hollmann, *Ber.*, **59**, 601, 1282 (1926)) on the basis of fairly complete chemical evidence, but reversal of the assignment for the dimethyl isomers has recently been suggested[6] on the basis of assumptions concerning the mechanism of condensation of methylhydrazine and β-keto acetals. If these later conclusions are valid, presumably many of the structural assignments made by von Auwers and others are open to question.

(6) D. M. Burness, *J. Org. Chem.*, **21**, 97 (1956).

(7) G. N. Walker and B. N. Weaver, *ibid.*, **26**, 4441 (1961).

(8) All new compounds gave satisfactory analytical values.

(9) J. A. Moore and J. Binkert, *J. Am. Chem. Soc.*, **81**, 6029 (1959).

(10) M. G. Reinecke, H. W. Johnson, Jr., and J. F. Sebastian, *Chem. Ind.* (London), 151 (1964).

ditional pairs of isomeric N-alkylpyrazoles of unambiguous structure become available.

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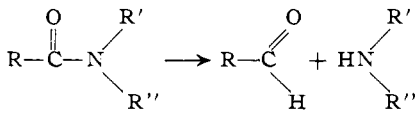
RECEIVED FEBRUARY 3, 1964

Chemical Cleavage of Proline Peptide Bonds

Sir:

We wish to report a reductive chemical cleavage of N-proline peptide bonds,[1a] utilizing lithium dissolved in methylamine.[1b] The reduction of tertiary amides is known to lead to the production of aldehydes[2-5] according to the following scheme.



N-proline peptide bonds are tertiary amides, therefore a similar cleavage would be expected to occur as follows.

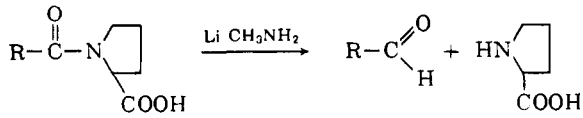


Table I lists the results obtained when a representative series of proline containing peptides were reduced by lithium dissolved in methylamine.

TABLE I

REDUCTIVE CLEAVAGE OF VARIOUS PROLINE PEPTIDES IN METHYLAMINE–LITHIUM SOLUTIONS

Peptide	Extent of cleavage, %	Method of determination
N-Acetyl-L-proline[c]	70	NIN[a]
L-Alanyl-L-proline[d]	66	NIN
Glycyl-L-proline[d]	71	NIN
Phenylpropionyl-L-proline[e]	70	NIN
Phenylpropionyl-L-prolyl-L-leucine[f]	62	PIP[b]
Val[5]-Hypertensin	51	PIP
Gramicidin "S,,	53	PIP
Glycyl-L-hydroxyproline	90	*p*-DAB[g]

[a] NIN = ninhydrin. [b] PIP = proline–imino peptidase treatment followed by colorimetric determination of proline. [c] D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, **193**, 81 (1951). [d] M. Bergmann, L. Zervas, H. Schleich, and F. Leinert, *Z. Physiol Chem.*, **212**, 72 (1932). [e] M.p. 107°. [f] M.p. 161–163°. [g] *p*-DAB = *p*-dimethylaminobenzaldehyde.

All the reductions were carried out under the following reaction conditions. A C-terminal proline dipeptide (0.5 mmole) was acetylated with acetic anhydride and dissolved in methylamine (30–40 ml.). N-methylacetamide (1 ml.) was added to minimize reduction of secondary amide–peptide bonds. The reaction mixture was cooled to −70° and an excess of metallic lithium was added. After 1 hr., a small amount of ammonium chloride was added to discharge the blue color of the mixture. The solvent was allowed to evaporate and the mixture was dissolved in water. Paper chromatography of the reduction mixture showed the presence of free proline which was determined colorimetrically.[6] The yield of proline was found to be 65–75% for all dipeptides treated.

Cleavage of the phenylpropionyl–L-proline bond in phenylpropionyl-L-prolyl-L-leucine was detected by paper chromatography using an authentic sample of L-prolyl-L-leucine as a marker and developing the paper chromatogram with acidic ninhydrin. The extent of this cleavage (62%) was determined using the specific exoenzyme L-proline imino peptidase[6] to cleave quantitatively the new N-terminal proline formed, followed by colorimetric determination of the free proline. The amount of C-terminal leucine (60%) found on enzymatic digestion was estimated by paper chromatography and quantitative ninhydrin assay.

The applicability of this method to larger molecules was tested by performing the cleavage on the synthetic decapeptide, Val[5]-Hypertensin, asp(NH_2)-arg-val-tyr-val-his-pro-phe-his-leu,[7] and on the cyclic peptide, Gramicidin "S,, -(val-orn-leu-phe-pro)$_2$-.[8]

Hydroxyproline peptides are cleaved similarly as shown by the high yield of hydroxyproline obtained by reductive cleavage of glycyl-L-hydroxyproline (Table I). The extent of cleavage was determined colorimetrically[9] after paper chromatography of the reaction mixture.

The use of this method for the detection of X–pro–Y sequences[10] (where X and Y represent amino acids) in cyclic peptides and proteins is now being studied.

Acknowledgment.—The authors thank Professor E. Katchalski for his interest in this work. This investigation was supported by grant No. AM-5098 from the National Institutes of Health, United States Public Health Service.

(1) (a) Preliminary attempts to cleave hydroxyproline bonds were reported by B. Witkop, "Advances in Protein Chemistry," Vol. 16, Academic Press, 1961, p. 235; (b) R. A. Benkeser, R. E. Robinson, D. M. Saure, and O. H. Thomas, *J. Am. Chem. Soc.*, **77**, 3230 (1955).

(2) F. Weygand and G. Eberhardt, *Angew. Chem.*, **64**, 458 (1952).

(3) A. J. Birch, J. Cymerman-Craig, and M. Slaytor, *Australian J. Chem.*, **8**, 512 (1955).

(4) H. C. Brown and B. C. Subba Rao, *J. Am. Chem. Soc.*, **80**, 5377 (1958).

(5) L. Birkofer and E. Frankus, *Chem. Ber.*, **94**, 216 (1961).

(6) S. Sarid, A. Berger, and E. Katchalski, *J. Biol. Chem.*, **234**, 1740 (1959); *ibid.*, **237**, 2207 (1962).

(7) This peptide was kindly supplied by Dr. R. Schwyzer of CIBA Ltd., Basle.

(8) This peptide was kindly supplied by Dr. M. M. Shemyakin, Institute for Chemistry of Natural Products, U.S.S.R. Academy of Science, Moscow.

(9) I. J. Bekhor and L. A. Bavetta, *Anal. Chem.*, **33**, 1807 (1961).

(10) S. Sarid and A. Patchornik, *Israel J. Chem.*, **1**, 63 (1963).

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RECEIVED JANUARY 24, 1964

Reduction of Acylated Aldono-γ-lactones to Aldofuranose Derivatives. A New Synthetic Pathway to Nucleosides[1]

Sir:

Since the majority of free sugars exist in the pyranose form, special methods must frequently be employed to

(1) This work is taken from a thesis submitted by Leon M. Lerner to the University of Illinois Graduate College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. It was supported in part by Training Grant No. GM-471 from the Division of General Medical Sciences of the United States Public Health Service and by Grant P-161 from the American Cancer Society.