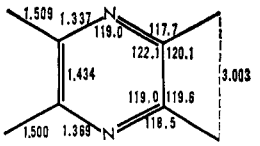

Figure 2. The bond lengths and angles of 2,3,5,6-tetramethylpyrazine.[7] Standard deviations in bond lengths and angles are about 0.01 Å and 0.6°, respectively.

nitrogen-containing ring that deviates significantly from true planarity. The distances of atoms 1, 2, 3, 4, 5, 10, 11, and 15 to the average plane of the ring are +0.009, +0.080, −0.080, −0.009, +0.067, −0.068, +0.446, and −0.380 Å, respectively (accurate to 0.005 Å).

This "twisted" ring may best be compared to a highly flattened boat with angles of about 3° deviation from planarity. The benzene ring and the conformation of atoms 11, 2, 3, and 15 of this molecule are practically planar. The slight twist in this molecule is probably a function of the best crystal fit. It is not due to steric repulsion between the *t*-butyl groups since inspection clearly shows that this twist does not significantly increase the distance between the *t*-butyl groups.

The C–N–C angle of 121° in Figure 1 is larger than the values found in pyrazine (115.1°),[8] 2,3,5,6-tetramethylpyrazine (119.0),[7] α-phenazine (116.3°),[9] and α-pyrazinamide (115.7°).[10]

It is clear that a combination of bond stretching and angle deformation in the plane of the ring is involved in relieving the strain in *o*-di-*t*-butyl aromatics. We have found no literature pertaining to the crystal structure of quinoxaline itself, and further detailed discussion of bond lengths appears premature at this stage.

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Synthesis of Peptides in Aqueous Medium. V. Preparation and Use of 2,5-Thiazolidinediones (NTA's). Use of the ^{13}C–H Nuclear Magnetic Resonance Signal as Internal Standard for Quantitative Studies

Sir:

The use of α-amino acid N-carboxyanhydrides (NCA's) in the synthesis of heteropeptides has been described.[1-3] The sulfur analogs[4] of the NCA's, the 2,5-thiazolidinediones (N-thiocarboxyanhydrides, NTA's), appeared worthy of study, especially since we found thiocarbamate salts to be more stable than the corresponding carbamate salts. We therefore[2] expected NTA's to give higher yields in peptide synthesis than NCA's. Furthermore, we found the optimal pH for NTA reactions to be lower (9.0–9.5) than for NCA reactions (10.2–10.5). The lower pH favors the desired aminolysis of an anhydride over hydrolysis[2] and this, too, should improve the yield of the desired product. That this proved indeed to be the case is illustrated by the preparation of alanylleucylphenylalanine. After acidification of the crude reaction mixtures, the crystalline tripeptide precipitated in 70.3% yield from the alanine NCA reaction but in 92% yield after use of the NTA.

A further distinction was seen between the NCA and the NTA of glycine. The α-amino acid N-carboxyanhydride of glycine is a unique NCA because of its marked propensity to form the isocyanate I (R = H) which competes with NCA's for nucleophiles to yield hydantoic acids rather than the desired peptides. We found that 2,5-thiazolidinedione[5-7] (II, R = H) (glycine NTA) has little tendency to undergo this side reaction. For example, the condensation of the NCA of glycine with phenylalanine at 0° and pH 10.2 gave the hydantoic acid in >20% yield,[2] but the reaction of II (1.5% excess) at 0° and pH 9.5 gave glycylphenylalanine in about 93% yield by direct analysis of the total reaction mixture on a Beckman-Spinco amino acid analyzer. The yield of the hydantoic acid was calculated to be <3%. Similarly, phenylalanylleucine afforded crystalline glycylphenylalanylleucine in 95 and 37% yields with the NTA (20% excess) and the NCA (10% excess), respectively.

NTA's of L-amino acids,[8] in contrast to L-NCA's, do not yield optically pure peptides. That this lack of optical purity may result from partial racemization in the preparation and in the use of these NTA's was suggested by comparison of the total amount of racemization (tlc[2] and leucine aminopeptidase studies) with the extent of racemization during peptide bond formation in aqueous medium (tritium incorporation[1] studies).

The reaction of L-alanine with methyl methylxanthate in aqueous alkali at 40–50° afforded the analytically pure[9] thionourethan: mp 113–115°; $\lambda_{max}^{CH_2Cl_2}$ 2.83, 3.30, 5.61, 5.80, and 6.63 μ; $[\alpha]^{25}_{589}$ −19.3°[9] (*c* 0.97, CH_2Cl_2). Treatment with PBr_3 in the presence of 1 equiv of imidazole gave the analytically pure NTA II (R = CH_3): mp 91–93°; $\lambda_{max}^{CH_2Cl_2}$ 2.81 and 5.82 μ,

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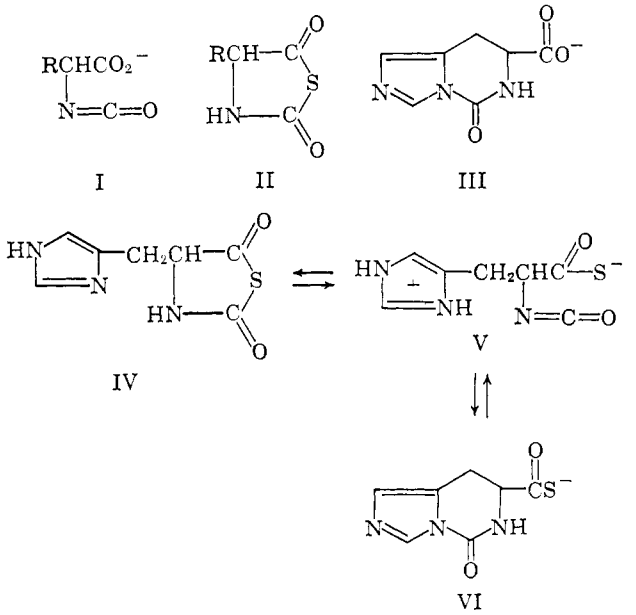
shoulder at 5.69 μ; $[\alpha]^{25}_{589}$ −1.64° (*c* 1.05, CH_2Cl_2). Treatment of a 1% excess of II (R = CH_3) with L-phenylalanine in a Waring Blendor at pH 9.5 and 0° gave the dipeptide in about 90% yield. In this case the optical purity of the dipeptide was determined by comparing the areas of the methyl doublets of the diastereoisomers in the nmr[10] spectrum of the crude lyophilized product in D_2O. We improved the sensitivity of the published analytical procedure[10] about tenfold by use of a ^{13}C–H satellite peak of the predominant L–L isomer as an internal standard. The dipeptide was thus found to contain 98.7 ± 0.3% of the L–L isomer. The ^{13}C–H satellite (abundance of ^{13}C = 1.11%) provides a convenient, reproducible internal reference standard for measurements in this range.

A striking chemical difference was also observed between the NCA and the NTA of imidazyl-unprotected histidine. We have found that the NCA of histidine cannot be used to prepare histidyl peptides because it rearranges to give III, doubtlessly *via* an intramolecular imidazole-catalyzed isocyanate formation. L-Histidine ethylthionourethan, mp 212° dec, $[\alpha]^{24}_{589}$ +24.0° (*c* 2.0, 0.1 *N* NaOH), on treatment with PBr_3 afforded the analytically pure NTA IV as the hydrobromide in 73% yield, $[\alpha]^{25}_{589}$ −7.0° (*c* 2%, methyl Carbitol). This compound, in contrast to the NCA, is useful for the rapid synthesis of imidazyl-unprotected histidyl peptides in aqueous medium possibly because the equilibrium between IV and V lies further to the left than the corresponding equilibrium of the oxygen analogs. Crystalline VI also yielded histidyl peptides, but the NTA IV permitted peptide bond formation at 0° in 3 min whereas higher temperatures and longer reaction times were required with VI. The NTA of histidine (IV) formed as much as 10% of the D-histidyl peptides, but it was often possible to purify the product. The reagent also proved advantageous whenever a simple procedure for the rapid introduction of imidazyl-unprotected histidine in aqueous medium was required, as, for example, in the preparation of reference compounds in connection with sequence determinations.



I II III

IV V

VI

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Thyrocalcitonin. II. Enzymatic and Chemical Sequence Studies

Sir:

The amino acid composition of the dotriacontapeptide thyrocalcitonin was reported from this laboratory in an earlier communication.[1] The assigned composition has been confirmed by two other laboratories.[2,3] From cleavage of the hormone with trypsin we have now obtained three fragments (1–14, 15–21, and 22–32) (Figure 1). Amino acid analyses[4] of these fragments accounted for all of the 32 amino acids (tryptophan was found after enzymatic cleavage) and were in agreement with those just reported by Kahnt, *et al.*[2] On the basis of degradation and synthetic studies summarized below, we report herein the sequence of amino acids 8–32.

Digestion of the hormone with trypsin and chymotrypsin (20 hr) followed by treatment with aminopeptidase M[5,6] (3 days) liberated Asx as asparagines and Glx as glutamic acid. Since the dotriacontapeptide is a monobasic acid,[1] the carboxyl group of the terminal amino acid (proline) is not free. Evidence for the presence of this proline as prolinamide and additional support for the formulation of Glx as glutamic acid was obtained by synthesis[7] of glycylprolylglutamylthreonylprolinamide. This pentapeptide amide was identical with a fragment (Chy-5) obtained by cleavage of the hormone with chymotrypsin. The synthetic[7] and the natural pentapeptides could be distinguished by electrophoresis and tlc from the isomeric pentapeptide glycylprolylglutaminylthreonylproline which we had also synthesized.[7] The presence of a disulfide bridge in thyrocalcitonin is suggested by the absence of free sulfhydryl groups in the hormone as shown by titration with 5,5′-dithiobis(2-nitrobenzoic acid).[8] Performic acid oxidation of fragment 1–14 gave 1.5 cysteic acid residues (theory = 2).

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