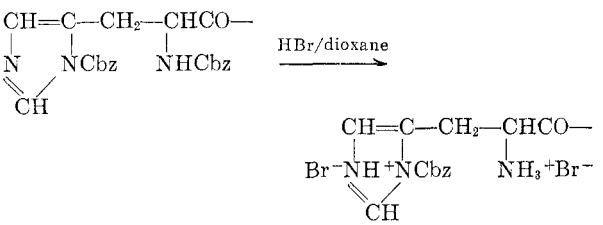
New Method for Synthesis of Peptides Containing Histidine

Sir:

In recent years it has been suggested that a histidine residue occupies a site of the biological activity in some naturally occurring peptides and proteins. Synthesis of histidine peptides is, therefore, very important for the study of protein biochemistry. However, the synthetic procedure has not been substantially improved for lack of the simple and satisfactory method to protect selectively the imidazole function of histidine.[1]

The purpose of this communication is to describe a new method for the synthesis of peptides containing histidine by selective blocking of the imidazole ring with a carbobenzoxy group.

Akabori *et al.*[2,3] have reported that $N(\alpha)$,N(Im)-dicarbobenzoxyhistidine is an excellent starting material in the synthesis of histidyl peptides. We have now found that the carbobenzoxy group linked at the imidazole-nitrogen (N(Im)-cbz) of histidine is surprisingly resistant to the treatment with hydrogen bromide in dioxane or in glacial acetic acid.



$N(\alpha)$,N(Im)-Di-cbz-histidine methyl ester hydrochloride (II) (m.p. 121–122.5° dec.[6]),[4] which was derived from di-cbz-histidine (I)[5] with thionyl chloride in methanol, and $N(\alpha)$,N(Im)-di-cbz-histidylphenylalanine methyl ester (III) (m.p. 136–137°) were treated with 35–40% (w/w)-hydrogen bromide in dioxane at room temperature for 30 min. to give N(Im)-cbz-histidine methyl ester dihydrobromide (IV) [94% yield, m.p. 167–167.5° dec., $\lambda_{max}^{CH_3OH}$ 234 mμ (ε 3140). *Anal.* Calcd. for $C_{15}H_{19}N_3O_4Br_2$: C, 38.75; H, 4.12; N, 9.04; Br, 34.4. Found: C, 37.23; H, 4.44; N, 9.43; Br, 35.03] and N(Im)-cbz-histidylphenylalanine methyl ester dihydrobromide (V) [99% yield, m.p. 126.5–127.5° dec., $\lambda_{max}^{CH_3OH}$ 234 mμ (ε 3300). *Anal.* Calcd. for $C_{24}H_{28}N_4O_5Br_2$: C, 47.15; H, 4.61; N, 9.15; Br, 26.15. Found: C, 46.87; H, 4.75; N, 9.05; Br, 26.26], respectively. These $N(\alpha)$-free derivatives are reactive to ninhydrin and their absorptions at 234 mμ were extremely depressed by adding sodium methoxide within a few minutes. This phenomenon shows the presence of an N-(Im)-cbz group.[6] On the other hand, these derivatives can react somewhat slowly with the Pauly reagent different from the disubstituted histidine analogs. This fact may show that the N(Im)-cbz group is very unstable in an alkaline medium in the absence of an $N(\alpha)$-substituting function.

IV or V could, however, be safely neutralized with dilute aqueous ammonia in methylene chloride at 0°. The resulting free esters were coupled with cbz-glycine by means of the carbodiimide method[7] to yield cbz-glycyl-N(Im)-cbz-histidine methyl ester (VI) [85% yield, m.p. 74–75.5°, $[\alpha]_D^{29}$ +24.5° (ethyl acetate), $\lambda_{max}^{CH_3OH}$ 236 mμ (ε 3755). *Anal.* Calcd. for $C_{25}H_{26}N_4O_7$: C, 60.7; H, 5.31; N, 11.3. Found: C, 60.61; H, 5.45; N, 11.43] and cbz-glycyl-N(Im)-cbz-histidylphenylalanine methyl ester(VII) [90% yield, m.p. 154–154.5°, $[\alpha]_D^{18}$ −8.6° (methanol), $\lambda_{max}^{CH_3OH}$ 236 mμ (ε 3655). *Anal.* Calcd. for $C_{34}H_{35}N_5O_8$: C, 63.7; H, 5.51; N, 10.9. Found: C, 63.69; H, 5.63; N, 10.84]. In a similar fashion we have obtained cbz-glycyl-N-(Im)-cbz-histidylleucine methyl ester (VIII) [82% yield, m.p. 152–152.5°, $[\alpha]_D^{18}$ −11.6° (methanol), $\lambda_{max}^{CH_3OH}$ 236 mμ (ε 3610). *Anal.* Calcd. for $C_{31}H_{37}N_5O_8$: C, 61.3; H, 6.14; N, 11.5. Found: C, 61.39; H, 6.31; N, 11.44], $N(\alpha)$, N(Im)-dicbz-histidyl-N-(Im)cbz-histidylphenylalanine benzyl ester(IX) [81% yield, m.p. 138–140°, $[\alpha]_D^{22}$ −17.9° (methanol), $\lambda_{max}^{CH_3OH}$ 236 mμ (ε 7830). *Anal.* Calcd. for $C_{52}H_{49}N_7O_{10}$: C, 67.1; H, 5.79; N, 10.5. Found: C, 67.05; H, 5.41; N, 10.69] and formyl-γ-methyl-glutamyl-N(Im)-cbz-histidylphenylalanine benzyl ester (X) [87% yield, m.p. 165–165.5° dec., $[\alpha]_D^{24}$ −12.1° (dimethylformamide), $\lambda_{max}^{CH_3OH}$ 236 mμ (ε 3415). *Anul.* Calcd. for $C_{37}H_{39}N_5O_9$: C, 63.7; H, 5.63; N, 10.02. Found: C, 63.79; H, 5.90; N, 10.36] which has a sequence contained in corticotropin and MSH. These histidine peptide derivatives (VI to X) are negative to both ninhydrin and the Pauly reagent and have moderate solubilities in common organic solvents.

The treatment of VII with hydrogen bromide/dioxane or with 2 equivalent amounts of sodium hydroxide gave glycyl-N(Im)-cbz-histidylphenyl-

(1) Benzyl [D. Theodoropoulos, *J. Org. Chem.*, **21**, 1550 (1956)] or trityl group [G. C. Stelakatos, D. M. Theodoropoulos, and L. Zervas, *J. Am. Chem. Soc.*, **81**, 2884 (1959)] has been used for the protection of the imidazole function but there are some defects for practical purposes.

(2) S. Akabori, K. Okawa, and F. Sakiyama, *Nature*, **181**, 772 (1958).

(3) F. Sakiyama, K. Okawa, T. Yamakawa, and S. Akabori, *Bull. Chem. Soc. Japan*, **31**, 926 (1958).

(4) Amino acids used are of L-configuration and all melting points are uncorrected.

(5) This could be crystallized in granules from a concentrated ethyl acetate solution [m.p. 90.5–92° dec., $[\alpha]_D^{19}$ +29.1° (ethyl acetate). *Anal.* Calcd. for $C_{22}H_{21}N_3O_6$: N, 9.93. Found: N, 9.86]. This crystal is readily soluble in methanol and from the solution is soon separated a second form of crystal containing one molecule of methanol, m.p. 105–107° dec.[3,6]

(6) A. Patchornik, A. Berger, and E. Katchalski, *J. Am. Chem. Soc.*, **79**, 6416 (1957).

(7) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

alanine methyl ester dihydrobromide (XI) [$\lambda_{max}^{CH_3OH}$ 234 mμ (ε 3640). *Anal.* Calcd. for $C_{26}H_{31}N_5O_6Br_2$: C, 46.7; H, 4.68; N, 10.01; Br, 23.9. Found: C, 45.73; H, 4.99; N, 10.40; Br, 22.59] or cbz-glycyl-histidylphenylalanine (XII) [77.5% yield, m.p. 200° dec., $[\alpha]_D^{30}$ + 15.4° (methanol). *Anal.* Calcd. for $C_{25}H_{27}N_5O_6$: C, 60.8; H, 5.52; N, 14.2. Found: C, 60.51; H, 5.65; N, 14.11], respectively. The *N*(Im)-cbz group may be removed also with catalytic hydrogenation.

From these results it has been demonstrated that *N*(Im)-cbz-histidine derivatives, which can be prepared by the simple and convenient procedure, are of potential utility as intermediates in the synthesis of histidine peptides.

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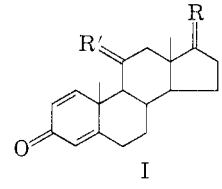
An Aromatization Reaction of A Cross-Conjugated Dienone System with Zinc

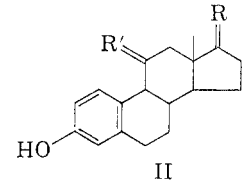
Sir:

We wish to report a new A-ring aromatization reaction of the dienone system with zinc under mild conditions. When refluxed with zinc in pyridine[1] or ethylene glycol, androstan-1,4,9(11)-triene-3,17-dione (Ia) undergoes A-ring aromatization with elimination of the angular methyl group to form Δ^9-estrone (IIa)[2] in excellent yield (75%). In a similar manner the treatment of 17α,21-dihydroxy-pregnan-1,4,9(11)-triene-3,20-dione acetate (Ib; 21-acetate) or its BMD derivative (Ic) in pyridine yielded, respectively, A-ring aromatic corticoids,[3] IIb 21-acetate, (yield: 35%), m.p. 210–212°, $[\alpha]_D^{20}$ +174° (dioxane), $\lambda_{max}^{CH_3OH}$ 263, 298 mμ (ε 18,000, 3,100), λ_{max}^{Nujol} 814 cm.$^{-1}$, (*Anal.* Calcd. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.08. Found: C, 71.48; H, 6.98); IIb, m.p. 248–250°; $[\alpha]_D^{20}$ +176° (dioxane); IIb, 3,21-diacetate, m.p. 188–190°, $[\alpha]_D^{20}$ +136° (chloroform), or IIc (yield: 72%), m.p. 247–248°, $[\alpha]_D^{20}$ +31° (dioxane), which was converted to IIb by acetic acid hydrolysis. 3-Keto-1,4,6-triene (Id) gave Δ^6-estrone (IId)[4] (yield, 10–15%) in the same reaction.

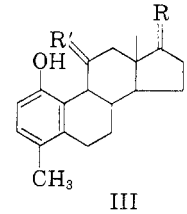
In the case of 3-keto-1,4-dienes or their C-11 substituted derivatives different rearrangement products were obtained. Treatment of Ie with zinc in pyridine provided a mixture of *p*-cresol type rearrangement product (IIIe)[5] (yield, 80%) and estrone (IIe) (yield, 4%). The 11β-hydroxy compound (If) yielded IIIf, m.p. 223–224°, $[\alpha]_D^{15}$ +249° (chloroform), $\lambda_{max}^{CH_3OH}$ 282–286 mμ (ε 2,340), methyl ether, m.p. 213–214°, $[\alpha]_D^{15}$ +303° (chloroform), which was confirmed by conversion to the IIIe methyl ether by dehydration and hydrogenation. However, the 11-keto compound (Ig) suffered rupture of C_9-C_{10} bond with concomitant A-ring aromatization to give the 9//10 seco compound (IV), m.p. 212–214°. It was identical in all respects with an authentic specimen of IV obtained from Ig by pyrolysis.[6]



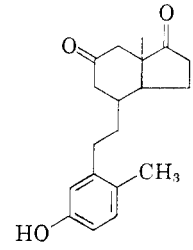
I



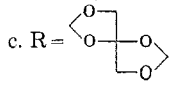
II



III



IV

a. R = O, R′ = H, $\Delta^{9(11)}$
b. R = α-OH, —CO—CH_2OH, R′ = H, $\Delta^{9(11)}$
c. R =  R′ = H, $\Delta^{9(11)}$
d. R = O, R′ = —H, —H, Δ^6
e. R = O, R′ = —H, —H
f. R = O, R′ = β-OH, —H
g. R = R′ = O

Santonin was converted to desmethyldesmotroposantonin (V) (yield, 40%), m.p. 223–227°, $[\alpha]_D^{20}$ +115° (chloroform), $\lambda_{max}^{CH_3OH}$ 286.5 mμ (ε 2,820), λ_{max}^{Nujol} 811 cm.$^{-1}$, NMR[7] τ, 7.60 ppm. (one benzenoid methyl) (*Anal.* Calcd. for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94. Found: C, 72.10; H, 6.93), acetate, m.p. 144–146° (*Anal.* Calcd. for $C_{16}H_{18}O_4$: C, 70.05; H, 6.61. Found: C, 70.08; H, 6.50.), by the same reaction in pyridine. The structure of V was confirmed by the palladium-charcoal dehydrogenation which led to 1-methyl-7-ethyl-

(1) Pyridine containing *ca.* 10 mole equivalents of water was used.

(2) B. J. Magerlein and J. A. Hogg, *J. Am. Chem. Soc.*, **80**, 2220 (1958).

(3) B. J. Magerlein and J. A. Hogg, *J. Am. Chem. Soc.*, **80**, 2226 (1958).

(4) St. Kaufman, J. Pataki, G. Rosenkranz, J. Romo, and C. D. Djerassi, *J. Am. Chem. Soc.*, **72**, 4531, 4534 (1950).

(5) A. S. Dreiding and A. Voltman, *J. Am. Chem. Soc.*, **76**, 537 (1954).

(6) B. J. Magerlein and J. A. Hogg, *Tetrahedron*, **2**, 80 (1958).

(7) τ values were calculated assuming τ chloroform (solvent) = 2.75 ppm.