Synthesis and Use of L-Histidine Benzylester

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Up to the present, histidine methylester[1] has been known to be an only actual example of the histidine esters. Izumiya[2] attempted the synthesis of histidine benzylester, but the benzylester in a pure form was not obtained by his procedure.

In this communication, crystalline histidine benzylester di-*p*-toluene sulfonate(I) was prepared in a good yield. A suspension of powdered L-histidine hydrochloride monohydrate (20 g.), *p*-toluene sulfonic acid monohydrate(44 g.) and benzyl alcohol (100 ml.) in chloroform (500 ml.) was refluxed for about 30 hr. in Wieland's[3] apparatus, in which silica gel and anhydrous sodium carbonate were used as desiccants. Distillation of chloroform, followed by addition of dry ether (350 ml.),

1) E. Fischer und L. H. Cone, *Ann.*, **363**, 107 (1908).
2) N. Izumiya and K. Makisumi, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **78**, 662 (1957).
3) T. Wieland, G. Ohnacker und W. Ziegler, *Ber.*, **90**, 194 (1957).

TABLE I

PEPTIDE-DERIVATIVES SYNTHESIZED FROM HISTIDINE BENZYLESTER

Substance	Solubility in dil. HCl	Yield (%)	Melting point (°C)	$[\alpha]_D^{30}$	Anal. (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Cbzo-Gly-L-His-OBz[a]	+	77.9	99~100.2	−6.8°[g]	63.29	5.54	12.84	63.01	5.73	12.43
// [b]		82.5	98.5~99.2	−5.5°[g]						
// [c]		45.8	99~99.5	−6.3°[g]						
Cbzo-L-Thr-L-His-OBz[b]	+++	66.5	137~137.5	−16.4°[h]	62.48	5.87	11.66	62.48	6.19	11.80
Cbzo-L-Phe-L-His-OBz[a]	−	68.5	118~118.5	−17.3°[h]	68.42	5.74	10.64	68.44	5.95	10.76
// [b]		53.3	117~118.5	−22.4°[h]						
Isovaleryl-L-His-OBz[d]	+	7.6	144	−23.6°[h]	65.63	7.04	12.76	65.79	7.29	12.72
H-Gly-L-His-OH HCl[e]		80.0	174~175 (dec.)[f]	+28.5°[i]						

(a), Mixed anhydride method (isovaleryl); (b), dicyclohexylcarbodiimide method; (c), azide method; (d), this substance was obtained as a byproduct from the dil. HCl extract of the reaction mixture of Cbzo-Phe-His-OBz (mixed anhydride method); (e), dried at 135° in vacuo; (f), corrected value; M. Hunt[5] reported m. p. 175° (corr.); (g), c 2, ethanol; (h), c 1, ethanol; (i), c 1, water; M. Hunt[5] reported $[\alpha]_D^{26}$ +25° (c 1, water).

Cbzo..... Carbobenzoxy, Bz..... benzyl.

gave a syrupy mass which was reprecipitated from chloroform and dry ether. The product was then crystallized gradually from a mixture of dry dioxane and dry acetone (1 : 2); colorless fine needles, weight 48~51 g. (85~90%), $[\alpha]_D^{30}$ −2.4° (c 4.7, water). This substance I was obtained in a hydrated state on recrystallization from a dioxane-acetone mixture containing a small amount of water. The hydrated crystals could be handled more conveniently than the anhydrous because the hydrated crystals were not so hygroscopic as the anhydrous ones. Almost all of the crystallization water, if necessary, could be dried off over phosphorous pentoxide in vacuo at 30°. These properties of the hydrated crystals, however, made difficult the quantitative determination of the crystallization water. This substance I sintered at 82~85° and decomposed at 225° after dryness in vacuo at ordinary temperature, and melted at 146~149° after dryness in vacuo at 135°.

Anal. Found (dried at 135°): C, 54.95; H, 5.48; N, 7.37. Found (hydrated crystals, dried in vacuo at 30°): C, 54.90; H, 5.46; N, 6.72. Calcd. for $C_{27}H_{31}O_8N_3S_2$: C, 54.99; H, 5.30; N, 7.13%.

Histidine methylester di-hydrochloride is hard to dissolve in usual organic solvents other than methanol or ethanol; and aqueous strong alkali or sodium methoxide is required for preparing the free methylester. On the other hand, the benzylester sulfonate I was considerably soluble in dry chloroform, and a solution of I and a calculated amount of triethylamine in chloroform could be used as a free benzylester solution for peptide synthesis. For example, carbobenzoxy glycyl histidine benzylester (II) was prepared as follows. A solution of mixed anhydride, which was prepared from carbobenzoxy glycine (1.05 g.), isovaleryl chloride (0.6 g.) and triethylamine (0.7 ml.) according to Vaughan and Osato's procedure[4], was allowed to react overnight with a mixture of I (3.54 g.) and triethylamine (1.70 ml.) in chloroform (15 ml.), and after usual treatment of the reaction mixture, the substance II was obtained in a yield of 1.7 g. The substance II was then hydrogenated in the presence of Palladium charcoal into glycyl histidine hydrochloride, and it was confirmed that the product obtained here was superior in specific optical rotation to the reported one[5] which was prepared from carbobenzoxy glycyl histidine methylester. The peptide-derivatives synthesized from the histidine benzylester are listed in Table I.

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4) J. R. Vaughan Jr., and R. L. Osato, *J. Am. Chem. Soc.*, **73**, 5553 (1951).
5) M. Hunt and V. du Vigneaud, *J. Biol. Chem.*, **127**, 46 (1939).