## L-Tyrosyl-L-phenylalanine

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The dipeptides derived from L-tyrosine and L-phenylalanine have been mentioned in the literature from time to time, mainly in connection with enzymatic hydrolysis studies.[1,2] In particular, L-tyrosyl-L-phenylalanine and various derivatives are referred to, but in only a few cases have any of these compounds been obtained pure and characterized. The synthesis of the pure dipeptide and several of its derivatives by conventional methods is described here.

### EXPERIMENTAL

*N,O-Biscarbobenzoxy-L-tyrosine.* There are considerable differences in the reported properties of this derivative.

Treatment of L-tyrosine in the normal way with excess carbobenzoxy chloride and alkali gave a noncrystalline white powder, m.p. 85–86°; $[\alpha]_D^{22}$ + 3.7° (*c* 10, acetic acid). Pannemann, Marx, and Arens[3] give m.p. 115–121° (indefinite); $[\alpha]_D^{24°}$ +4.5° (*c* 10, acetic acid), while Katchalski and Sela[4] report colorless needles, m.p. 117°; $[\alpha]_D^{20°}$ −5° (*c* 10, acetic acid).

*N,O-Biscarbobenzoxy-L-tyrosyl-L-phenylalanine.* An ice cold solution of 3.0 g. of *N,O*-biscarbobenzoxy-L-tyrosine and 0.7 g. of triethylamine in 14 ml. of tetrahydrofuran was treated dropwise with 0.9 g. of isobutyl chlorocarbonate. After stirring for 5 min. a solution of 1.1 g. of L-phenylalanine in 6.4 ml of *N* NaOH was added in one lot. The mixture was stirred for 30 min., and acidified. The oil which separated soon solidified and was recrystallized from ethyl acetate–hexane, yield 3.1 g. (78%) m.p. 179–181°. Recrystallization from ethanol raised the m.p. to 182–184°. $[\alpha]_D^{21°}$ +6.1° (*c* 1, acetic acid).

*Anal.* Calcd. for $C_{34}H_{32}O_8N_2$: C, 68.4; H, 5.4; N, 4.7. Found: C, 68.6; H, 5.6; N, 4.7.

*N-Carbobenzoxy-L-tyrosyl-L-phenylalanine.* A dried ethereal solution of the azide obtained in the usual way[5] from 6.2 g. of *N*-carbobenzoxy-L-tyrosyl hydrazide was mixed with an ether solution of ethyl L-phenylalanate obtained from 4.3 g. of the hydrochloride. The mixed solution was kept overnight at 0° and the precipitated crude ethyl *N*-carbobenzoxy-L-tyrosyl-L-phenylalanate filtered and washed with ether. The yield of amorphous solid was 5.0 g. (55%).

Treatment of 2.0 g. of this ester with 9.2 ml. of *N* sodium hydroxide and 5 ml. of dioxane, followed by acidification, gave *N*-carbobenzoxy-L-tyrosyl-L-phenylalanine, which was recrystallized from aqueous ethanol. Yield, 1.7 g. (90%), m.p. 175–176°; $[\alpha]_D^{22°}$ +2.1° (*c* 1, *N* sodium hydroxide).

*Anal.* Calcd. for $C_{26}H_{26}O_6N_2$: C, 67.5; H, 5.6; N, 6.0. Found: C, 67.6; H, 5.8; N, 6.0.

The compound was also obtained in low yield (11%; m.p. 174.5–175.5°) by a mixed anhydride coupling of *N*-carbobenzoxy-L-tyrosine with L-phenylalanine using isobutyl chlorocarbonate.

*L-Tyrosyl-L-phenylalanine.* A solution of 3.4 g. of *N,O*-biscarbobenzoxy-L-tyrosyl-L-phenylalanine in 30 ml. of 4*M* hydrogen bromide in glacial acetic acid was warmed to 70° for 10 min., cooled and diluted with ether. The oily precipitate was dissolved in water and the solution neutralized to litmus with aqueous potassium carbonate. On addition of ethanol the dipeptide separated slowly as colorless needles of the monohydrate, yield 0.8 g. (42%), m.p. 308–310° dec.; $[\alpha]_D^{21°}$ +17.7° (*c* 0.5, 2*N* hydrochloric acid). The water of hydration was very firmly bound.

*Anal.* Calcd. for $C_{18}H_{22}O_5N_2$: C, 62.3; H, 6.4; N, 8.1. Found: C, 62.4; H, 6.5; N, 8.3.

The dipeptide was also obtained by treating *N*-carbobenzoxy-L-tyrosyl-L-phenylalanine with hydrogen bromide in acetic acid at room temperature. Recrystallized from water, m.p. 310–312° dec.; $[\alpha]_D^{22.5°}$ +17.2° (*c* 0.267, 2*N* hydrochloric acid).

*N-Formyl-L-tyrosyl-L-phenylalanine.* The ethyl ester[6] was obtained in very poor yield by the *N,N'*-dicyclohexylcarbodiimide[7] coupling of *N*-formyl-L-tyrosine and ethyl L-phenylalanate. It was repeatedly recrystallized from aqueous ethanol, m.p. 174–175°; $[\alpha]_D^{21°}$ +2.4° (*c* 1, ethanol). Treatment of 50 mg. of the ester with 0.4 ml. of *N* sodium hydroxide, followed by acidification gave 40.6 mg. (88%) of the product, which formed colorless needles from aqueous ethanol, m.p. 247–248° dec.

*Anal.* Calcd. for $C_{19}H_{20}O_5N_2$: N, 7.9. Found: N, 7.8.

This derivative was also obtained by warming 100 mg. of L-tyrosyl-L-phenylalanine with 0.6 ml. of a 2:1 mixture of 90% formic acid and acetic anhydride. It was recrystallized from aqueous ethanol, m.p. 246–247° (alone and mixed with above product), yield 35 mg. (30%).

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(1) K. Blau, and S. G. Waley, *Biochem. J.*, **57**, 538 (1954).
(2) L. E. Baker, *J. Biol. Chem.*, **193**, 809 (1951).
(3) H. J. Pannemann, A. F. Marx, and J. F. Arens, *Rec. trav. chim.*, **78**, 487 (1959).
(4) E. Katchalski and M. Sela, *J. Am. Chem. Soc.*, **75**, 5284 (1953).
(5) C. R. Harrington and R. V. Pitt-Rivers, *Biochem. J.*, **38**, 417 (1944).
(6) R. W. Roeske, unpublished work.
(7) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

## An Unusual Transfer Reaction in the Steroid Series

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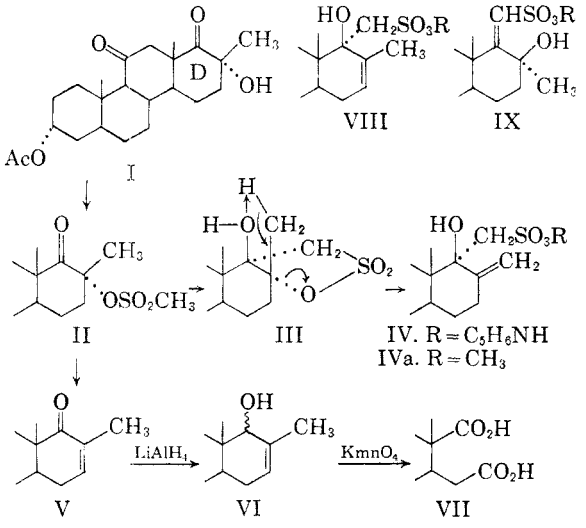
The unusual reactivity of the ketolic system, 3α-acetoxy-17α-hydroxy-17β-methyl-D-homo-5β-androstane-11,17a-dione (I), has been referred to recently.[1] The reaction of this substance with methanesulfonyl chloride in pyridine at 0° affords, in addition to the normal mesylate (II),[1a] an isomeric substance, m.p. 142–144° (25–30%), exhibiting strong hydroxyl absorption in the infrared.

(1) (a) N. L. Wendler, D. Taub, S. Dobriner, and D. K. Fukushima, *J. Am. Chem. Soc.*, **78**, 5027 (1956) Footnote 14. See also (b) N. L. Wendler and D. Taub, *J. Org. Chem.*, **23**, 953 (1958).

This compound consequently has been formulated as the inner aldol (III).[2] On refluxing in pyridine (conditions which convert II→V[1a]), III was transformed to the pyridinium salt of a sulfonic acid, assigned structure IV on the basis of the following evidence:

The pyridinium salt was water soluble and could be titrated with perchloric acid to give an equivalent weight in excellent agreement with that calculated for IV or an isomer. Treatment of the pyridinium salt in methanol with ethereal diazomethane converted it to the corresponding methyl ester IVa. This ester exhibited no maximum in the ultraviolet, but possessed OH absorption at 2.83 $\mu$ as well as intense double bond absorption at 6.04 $\mu$ in the infrared. Although this ester was essentially inert to neutral permanganate, it did react slowly with osmium tetroxide thereby chemically confirming the presence of a double bond.




The lack of ultraviolet absorption is inconsistent with the isomeric possibility IX; the data are incompatible as well with the alternate structure VIII as this double bonded type, *e.g.* VI, exhibits no double bond absorption in the infrared under comparable conditions of measurement (see Experimental) and is readily oxidized by neutral permanganate to the etiobilianic acid VII.[1] Finally, the NMR spectrum was consistent with IVa and, by establishing the absence of any D-ring methyl group, clearly eliminated structures VIII and IX.[3]

The formation of the exomethylenic system IV would appear to constitute an example of the Arnold-Schinz mechanism of cyclicly assisted dehydration.[4]

## EXPERIMENTAL[5]

*Reaction of 3α-acetoxy-17α-hydroxy-17β-methyl-D-homo-5β-androstane-11,17a-dione* (I) *with methanesulfonyl chloride.* A solution of 4.1 g. of I in 15 cc. of anhydrous pyridine was treated at 0° with 4 cc. of methanesulfonyl chloride and allowed to stand at 0° for 16 hr. The reaction product was treated with ice and ether. The ether extract was washed successively with dilute aqueous hydrochloric acid, potassium bicarbonate, and sodium chloride solution. The washed ether solution was dried over magnesium sulfate, filtered, and evaporated to the point of incipient turbidity. This solution deposited 1.5 g. of III over a period of several days. Recrystallization from acetone-hexane afforded III as slender prisms, m.p. 142–144° dec., $\lambda_{max}^{Nujol}$ 3 $\mu$ (OH); 5.85 $\mu$ (C=O); 5.8 $\mu$, 8 $\mu$ (OAc), 7.4 $\mu$, 8.6 $\mu$ ($OSO_2$).

*Anal.* Calcd. for $C_{24}H_{36}O_7S$: C, 61.54; H, 7.70; S, 6.84. Found: C, 61.65; H, 7.70; S, 6.49.

*Pyridinium salt* (IV). A solution of 3 g. of III in 50 cc. of pyridine was refluxed for 2 hr. and evaporated to dryness *in vacuo.* The residue was crystallized from acetone-ether, 2.8 g., m.p. 145–150° $\lambda_{max}^{Nujol}$ 2.97 $\mu$ (OH/NH); 5.89 $\mu$ (C=O), 5.79, 8 $\mu$ (OAc), 6.1, 6.42 $\mu$ (Pyridine).

*Anal.* Calcd. for $C_{29}H_{41}O_7NS$: C, 63.62; H, 7.49; N, 2.74; S, 5.86; Eq. wt., 547. Found: C, 63.88; H, 7.17; N, 2.57; S, 6.01; Eq. wt., 533.

*Methyl ester* (IVa). Treatment of the pyridinium salt (IV) in methanol solution with an excess of ethereal diazomethane afforded the methyl ester (VIa) crystallized from ether, m.p. 180.5–182°, $\lambda_{max}^{CHCl_3}$ 2.83 $\mu$ (OH); 5.8–5.85 $\mu$, 8 $\mu$ (C=O, OAc); 7.3 $\mu$, 8.5–8.6 $\mu$ ($OSO_2$), 6.04 $\mu$ (C=C).

*Anal.* Calcd. for $C_{25}H_{38}O_7S$: C, 62.24; H, 7.88; S, 6.64. Found: C, 62.46; H, 7.85; S, 6.71.

The above ester (100 mg.) was recovered essentially unchanged after oxidation with potassium permanganate (200 mg.) in acetone (15 cc.) at 25° for 2 hr. or after refluxing for 1 hr.

*$\Delta^{16}$-17-Methyl-D-homo-5β-androstene-3α,11β,17a-triol* (VI). A solution of 100 mg. of the $\Delta^{\alpha,\beta}$ ketone (V)[1] in 20 cc. of ether was reduced with 200 mg. of lithium aluminum hydride at room temperature for 5 hr. The isolated triol (VI) crystallized from ether, m.p. *ca.* 280° $\lambda_{max}^{Nujol}$ 2.81, 3.03 $\mu$ (OH).

*Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.45; H, 10.18. Found: C, 75.49; H, 9.87.

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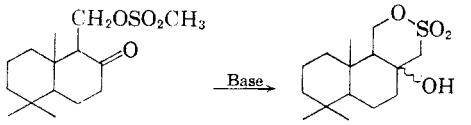
(2) E. Romann, A. J. Frey, P. A. Stadler, and A. Eschenmoser, [*Helv. Chim. Acta.*, **40**, 1900 (1957) Footnote 13] have reported a similar reaction:




We are grateful to Professor R. B. Woodward for calling this reference to our attention.

(3) The authors are grateful to N. R. Trenner and B. Arison for the NMR determination.

(4) R. T. Arnold, *Helv. Chim. Acta.*, **32**, 134 (1949); H. Schinz and G. Schäppi, *Helv. Chim. Acta.* **30**, 1483 (1947).

(5) Melting points were taken on a micro hot stage and are corrected. Infrared spectra were determined on a Baird Associates Infrared Spectrophotometer.

## Configuration and Conformation of 3-Bromohesperetin Triacetate. Dimorphs of Hesperetin Triacetate[1,2]

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Hesperetin (I), the aglycon of the important glycoside hesperidin, has been investigated rather