threo- and *erythro*-β-Hydroxy-DL-aspartic acids

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Received May 1, 1969

An improved procedure for the synthesis of *threo*- and *erythro*-β-hydroxy-DL-aspartic acids is described. An nuclear magnetic resonance study of both diastereomers indicated that the *threo* isomer exists primarily as the conformer having *anti* carboxyl groups while the *erythro* compound prefers a conformation with *gauche* carboxyl functions. Possible reasons for this anomaly are presented.

Canadian Journal of Chemistry, 47, 4363 (1969)

During the pursuit of intermediates useful in the synthesis of cycloserine analogs, we became interested in the diastereomers of racemic β-hydroxyaspartic acid (HAA). The synthesis of these isomers was carried out using a reaction path described by Miller (*1*); i.e., the treatment of *cis*- and *trans*-epoxysuccinic acids with ammonia, with some considerable modification of the actual procedure. The sodium salt of *cis*-epoxysuccinic acid, which was obtained directly from the oxidation of maleic acid, was treated with concentrated ammonium hydroxide without the troublesome prior conversion to the free acid as was done by the early workers. This resulted in a considerable saving of time and an increased yield of the *threo* amino acids. The *erythro* isomer was also prepared by this modified procedure, and, even though the product had the expected physical properties, we found that it was impure. The melting points of these highly polar amino acids are very poor indices of purity since partial decomposition always occurs during heating. Dimethyl sulfite esterification of the recrystallized *erythro* amino acid gave a dimethyl ester which consistently contained excess nitrogen by elemental analysis. Apparently, an ammonium salt was coprecipitated with the acid when it was isolated from the amination reaction mixture by acidification. Since recrystallization failed to remove this, the amino acid was placed on a strongly basic anion exchange resin (Amberlite IRA-400) and eluted with acetic acid. Pure erythro-DL-HAA was obtained which gave derivatives having the correct nitrogen content.[3]

Having the pure diastereomers in hand, we investigated their nuclear magnetic resonance (n.m.r.) spectra in D_2O where only the H_α, H_β protons were detectable. Previous investigations of amino acid n.m.r. spectra (*3*) indicated that the size of the vicinal coupling constant, $J_{\alpha,\beta}$, might allow us to estimate which rotational conformation of HAA is preferred. Both cysteine and histidine (*3d*) had $J_{\alpha,\beta}$ values consistent with the Karplus relationship (*4*), but those for β-hydroxy amino acids, threonine, and *allo*-threonine are somewhat anomalous (*3f*); i.e., assuming that the preferred rotomer will have the carboxyl and β-substituents *anti*, the *threo* isomer should show $J_{\alpha,\beta} \sim 3$ and *erythro* isomer should have $J_{\alpha,\beta} \sim 11$ c.p.s., whereas the observed values are 3.8 and 3.6 c.p.s., respectively. A similar "anomaly" was reported in connection with the *N*,β-dimethylleucines and *N*-methylisoleucine (*5*). Reports by Pachler (*3a,b*) on aspartic acid and by Alberty and co-workers (*6a*), Gawron and co-worker (*6b*), and Pachler (*3a*) on malic acid indicated, however, that these dicarboxylic acids do indeed exist preferably with the carboxyl groups in the *anti* conformation. We were led, therefore, to expect that *threo*-HAA might have a small $J_{\alpha,\beta}$ (see TA, Chart 1) while that of the *erythro* isomer would be large (see EA, Chart 1). Tables I and II show that *erythro*-HAA does not conform to these expectations, but *threo*-HAA does.

When the staggered conformations for these amino acids (T = *threo*, E = *erythro*, R = H) are examined (Chart 1), the small $J_{\alpha,\beta}$ of the *threo* acid suggests that conformers TA and TB having H_α and H_β *gauche* are very largely preferred over TC. It follows from the conformer populations (Table II), that the equilibrium constant for the formation of TA + TB from TC is approximately 99, which corresponds

[1]Abstracted from the Master of Science thesis presented to the University of Georgia Graduate School by C. W. Jones, III, in June, 1968.

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[3]Early workers who obtained *erythro*-DL-HAA by acidification of its ammoniacal solutions undoubtedly had an impure product; cf. (*2*).

TABLE I

$J_{\alpha,\beta}$, ν_α, ν_β, and conformer populations at various pH's for *erythro*-β-hydroxy-DL-aspartic acid

				Conformer populations*	
pH	$J_{\alpha,\beta}$(c.p.s.)	ν_α†	ν_β†	EA	EB + EC
1.00	2.8	480	492	0.02	0.98
2.96	3.0	426	459	0.04	0.96
3.65	3.1	424	453		
4.05	3.2	415	445	0.05	0.95
4.65	3.3	409	437		
6.82	3.4	407	432	0.07	0.93
7.75	3.4	404	432		
8.18	3.5	402	431		
9.25	3.6	392	428	0.09	0.91
9.70	3.8	357	411		
10.20	4.0	348	409	0.13	0.87
12.85	4.3	343	409		
13.30	4.3	343	409	0.15	0.85

*Calculated by the method of K. Pachler (9) using $J_g = 2.6$ and $J_{anti} = 13.6$. When these couplings were calculated using the electronegativities of the substituents, $J_g = 2.2$ and $J_{anti} = 11.4$. The use of these values made only slight changes in the rotomer populations.
†100 Hz values.

TABLE II

$J_{\alpha,\beta}$, ν_α, ν_β, and conformer populations at high and low pH for *threo*-β-hydroxy-DL-aspartic acid

				Conformer populations*	
pH	$J_{\alpha,\beta}$(c.p.s.)	ν_α†	ν_β†	TC	TA + TB
1.00	2.7	468	504	0.01	0.99
13.20	2.7	359	434	0.01	0.99

*See Table I.
†100 Hz values.

(7) to a ΔG of -2.72 kcal/mole. In order to calculate the enthalpy change for this equilibrium, we must take into account the fact that *two* of the possible conformers have a small $J_{\alpha,\beta}$ while only one has a large $J_{\alpha,\beta}$. The rotamers having the small coupling are favored probability-wise and ΔS for the equilibrium can be equated (7) to R ln 2. Using similar arguments for the *erythro* isomer, the thermodynamic parameters (see Table III) for the equilibrium formation of EB + EC (conformations with gauche protons) from EA were also calculated. These results show that the EB + EC mixture is strongly favored even though both of these conformations have *gauche* carboxyl groups. The overwhelmingly favored conformation (TA) of the *threo* isomer[4] has *anti* carboxyl functions and the question as to why the *anti* conformation is favored in one isomer and disfavored in the other is complex and cannot be completely explained at this time. As the pH of the solutions is increased, increasing electrostatic repulsion between the negatively charged carboxyl groups will cause the anti-conformers (EA and TA) to be favored. Since $J_{\alpha,\beta}$ of the *threo* isomer underwent essentially no change with pH, it most probably exists in both acid and base as the *anti* conformer, TA. The *erythro* compound should show a considerable increase in $J_{\alpha,\beta}$ as the pH rises since conformer EA should become more prevalent. The small change from 2.8 to only 4.3 c.p.s. concurrent with a pH increase of approximately 12 units shows that the concentration of EA does increase, but not to a predominant level. An n.m.r. study of both *threo*- and *erythro*-HAA in basic D_2O solution at temperatures between 30 and 90° showed essentially no change in $J_{\alpha,\beta}$ consistent

[4]TA is doubly favored because it allows a hydroxyl–amine hydrogen bond but lacks the carboxyl–carboxyl *gauche* interaction of TB.

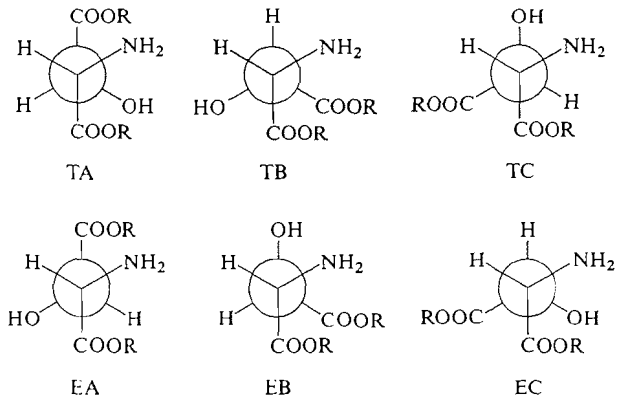


CHART 1

TABLE III
Thermodynamic parameters

	p*H*	*K*	Δ*G*(kcal/mole)	Δ*H*(kcal/mole)
threo-HAA TC ⇌ TA + TB	1.00 and 13.20	99	−2.72	−2.31
erythro-HAA	2.96	24	−1.88	−1.46
EA ⇌ EB + EC	13.30	5.75	−1.03	−0.62

with the small Δ*H* associated with these equilibria. The addition of Cd^{++} and Zn^{++} to solutions of these isomers also caused no change in $J_{\alpha,\beta}$ but did cause some shift in ν_α and ν_β.

In the course of the work on *erythro*-HAA, we determined pK_{a_1} (4.05) and pK_{a_2} (9.75) for the equilibria shown in Scheme 1. A plot of ν_α and ν_β vs. p*H* as shown in Fig. 1 allowed assignment of the more downfield doublet to H_β since this absorption underwent the larger diamagnetic shift (22 vs. 15 c.p.s.) during the first ionization, while H_α shifted more (44 vs. 17 c.p.s.) during the second. None of this information could be obtained for *threo*-HAA due to its very low solubility in water at ambient temperature.

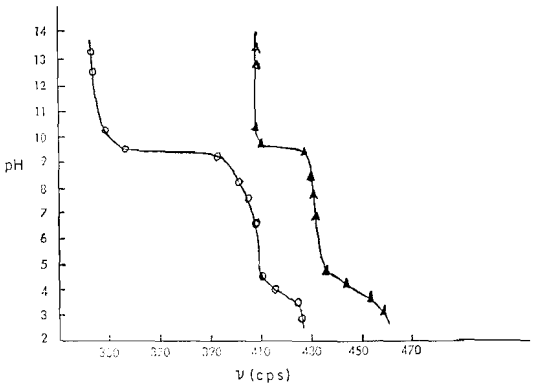


FIG. 1. Plot of p*H* versus ν(c.p.s., 100 Hz values) for *erythro*-HAA. ○—○, H_α values; ▲—▲, H_β values.

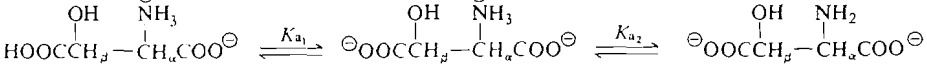
Experimental

Spectral Measurements

The proton magnetic resonance spectra were recorded using a Varian HA-100 high resolution n.m.r. spectrometer. Chemical shift measurements were made by the usual side band technique. Sodium-3-(trimethylsilyl)-propanesulfonate (tms*) was the internal reference compound. The ambient temperature of the sample compartment was 29 ± 2 °C. The precision of the chemical shift measurement was estimated to be better than ±0.01 p.p.m.

Measurement of pK_a Values

Measurements of p*H* were made at room temperature using a Leeds and Northrup Model No. 7403-A1 line operated expanded scale p*H* meter equipped with Model 124 138 miniature electrode assembly and standardized



SCHEME 1

using National Bureau of Standard buffers. No corrections were made for glass electrode errors at either p*H* extreme. The pK_a values of the compounds in D_2O were determined by potentiometric titration, using a semi-automatic recording device previously described. The titrant used was standard 6 *M* CO_2-free potassium deuteroxide solution. The KOD solution was prepared by reacting reagent grade potassium metal with CO_2-free D_2O (99.8%) in a nitrogen atmosphere. A layer of ether covered the D_2O to slow the reaction rate. The ether was removed by warming the KOD solution with hot water while passing a stream of nitrogen over the surface.

Infrared (i.r.) spectra were recorded on either a Perkin–Elmer Infracord Model 137 or Model 237-B. Melting points were taken on a Nalge–Axelrod apparatus and are corrected. Radial paper chromatography was carried out on 31-cm Whatman No. 1 circles having a 1 cm center hole. Compounds were visualized using ninhydrin (N). A substance having an R_f of 0.5 in the MPW system and located with ninhydrin is reported as R_f^{MPW} 0.5 (N). The solvent systems were as follows: MPW was methyl ethyl ketone:pyridine:water, 20:5:8; BAW was butanol:-acetic acid:water, 4:1:5 (upper phase); MAW was methyl ethyl ketone:acetic acid:water, 20:5:8.

erythro-β-*Hydroxy*-DL-*aspartic Acid*

A solution of 79.0 g (0.60 mole) *trans*-epoxysuccinic acid (8) in 2 l of concentrated ammonium hydroxide was heated to 40–50° for 25 h. The solution was evaporated to dryness on a rotary evaporator to remove residual ammonia and the residue was dissolved in sufficient water to make a 3% solution (w/v). The solution was placed on a column (6.5 × 65.0 cm) containing 2 pounds of Amberlite IRA-400 anion exchange resin (OH^- cycle) and eluted with 10% acetic acid. The combined acidic eluate was evaporated to dryness using a rotary evaporator and the resulting amino acid was crystallized from a 1:1 water:ethanol mixture giving 41.0 g (46%); i.r. (KBr) 3436 (O—H), 1710, and 1690 cm^{-1} (C=O); R_f^{MPW} 0.20 (N), R_f^{MAW} 0.64 (N).

threo-β-*Hydroxy*-DL-*aspartic Acid*

The disodium salt of *cis*-epoxysuccinic acid was prepared from 116 g (1 mole) maleic acid by the method of Payne and Williams (8). The crude salt was dissolved in 3.5 l conc. ammonium hydroxide and the solution was heated to 40–50° for 10 h. The reaction mixture was concentrated *in vacuo* to a heavy syrup and 700 ml of water was added. The solution was acidified to p*H* 3 with concentrated hydrochloric acid and kept at 5° for 12 h. The first crop, weighing 113.4 g, was filtered, washed with cold water, and dried *in vacuo*, over phosphorous pentoxide. The second crop, weighing 18.8 g, was obtained from the concentrated filtrate after cooling. The combined crude product was recrystallized from water giving a total of 66.0 g (44%) of *threo*-HAA; i.r. (nujol) 2.95 (O—H), 5.2, and 6.0 μ (C=O); R_f^{MPW} 0.21 (N), R_f^{BAW} 0.20 (N), R_f^{MAW} 0.42 (N).

Anal. Calcd. for $C_4H_7NO_2$: C, 32.23; H, 4.73; N, 9.38. Found: C, 32.19; H, 4.89; N, 9.35.

erythro-β-*Hydroxy*-DL-*aspartic Acid Dimethyl Ester Hydrochloride*

A solution of dimethylsulfite in methanol was prepared by the addition of 34 ml (0.48 mole) thionyl chloride to 230 ml of ice-cold methanol stirred magnetically. To this solution was added 23.0 g (0.15 mole) *erythro*-HAA and the mixture was refluxed 6 h. The solution was evaporated on a rotary evaporator, and the residue was redissolved in ca. 100 ml of methanol and the solution again evaporated to dryness. This process was repeated twice to remove excess hydrogen chloride. The resulting solid was pumped mechanically for 2 h and dissolved in a minimum amount of hot methanol. The solution was cooled and diluted with ether until crystals appeared. Slow dilution with ether as crystallization proceeded gave 25.0 g (76%) of crude *erythro*-β-hydroxy-DL-aspartic acid dimethyl ester hydrochloride; m.p. 148–151°; i.r. (KBr) 3240 ($\overset{+}{N}H_3$), 1750 cm^{-1} (C=O); n.m.r. (D_2O) δ 4.85 and 4.75 (d, 2, J = 2.8 Hz, H_β, H_α), and 3.83 p.p.m. (s, 6, —OCH_3); R_f^{MPW} 0.90 (N). A sample recrystallized three times from methanol–ether for analysis melted at 152–153°.

Anal. Calcd. for $C_6H_{12}ClNO_5$: C, 33.73; H, 5.66; N, 6.56. Found: C, 33.49; H, 5.78; N, 6.71.

threo-β-*Hydroxy*-DL-*aspartic Acid Dimethyl Ester Hydrochloride*

The procedure for synthesis of the *threo* ester hydrochloride was the same as that described for the *erythro* isomer; yield 31.7 g (96%); m.p. 134–136°; i.r. (nujol) 3.05 (O—H), 3.20 ($\overset{+}{N}H_3$), 5.70, and 5.75 μ (C=O); n.m.r. (D_2O) δ 4.91 and 4.48 (d, 2, J = 3.0 Hz, H_α, H_β) 3.88 (s, 3, —OCH_3), and 3.84 p.p.m. (s, 3, —OCH_3); R_f^{MPW} 0.91 (N), R_f^{MAW} 0.78 (N). Recrystallization of the crude product from methanol–ether gave an analytical sample.

Anal. Calcd. for $C_6H_{12}ClNO_5$: C, 33.73; H, 5.66; N, 6.56; Cl, 16.60. Found: C, 33.95; H, 5.69; N, 6.85; Cl, 16.53.

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