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SHORT COMMUNICATION

CONFIGURATION OF L-γ-ETHYLIDENEGLUTAMIC ACID FROM *GUILANDINA CRISTA**

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Key Word Index—*Guilandina crista*; Leguminosae; L-γ-ethylideneglutamic acid; configuration; use of NMR shift reagent.

Abstract—Using an NMR shift reagent, it has been established that the configuration about the double bond in naturally occurring L-γ-ethylideneglutamic acid is *cis*.

INTRODUCTION

CHROMATOGRAPHIC analysis of seed extracts of *Guilandina crista* (Leguminosae) showed that the principle free amino acid of the seeds of this plant is *erythro*-L-γ-methylglutamic acid which has previously been found in species of many diverse genera including *Phyllitis scolopendrium*,[1] *Polygala vulgaris*,[2] two species of *Lathyrus*[3] and many species of Liliaceae.[4] The seeds of *Guilandina crista* also contained lower concentrations of L-γ-methyleneglutamic acid which is not uncommon in plants[5–7] and L-γ-ethylideneglutamic acid which has been isolated previously from *Tulipa gesneriana*[8] and *Tetrapleura tetraptera*.[7]

Earlier attempts to establish whether naturally occurring L-γ-ethylideneglutamic acid had a *cis* or *trans* configuration were hampered by the availability of only one isomeric form (the isolated compound) for examination. A lack of broadening in the NMR signal given by the vinyl proton suggested that the compound might have a *trans* configuration,[8] while its NMR spectrum, molecular extinction in UV light and pK_a values resembled those of *cis*-ethylidenesuccinic acid and tiglic acid rather than those of *trans*-ethylidenesuccinic acid and angelic acid, suggesting that the *cis* configuration was the more probable.[7]

The recent development of NMR shift reagents has provided a new approach to this structural problem and the present paper describes the isolation of γ-ethylideneglutamic acid from seeds of *Guilandina crista*, the establishment of its identity with the compounds found in the other two species and the unequivocal determination of its configuration as *cis* using the NMR shift reagent $Eu(fod)_3$.[9]

* Based on a paper presented at the 11th Annual Meeting of the Phytochemical Society of North America at Monterrey, Mexico, October 1971.

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RESULTS AND DISCUSSION

L-γ-Ethylideneglutamic acid was isolated from seeds of *Guilandina crista* and found to be identical in all respects with the compound isolated from *Tulipa gesneriana*,[8] and *Tetrapleura tetraptera*.[7]

The unsaturated amino acid was esterified and the NMR spectrum of the dimethyl ester determined. $Eu(fod)_3$, [*tris* (1,1,1,2,2,3,3, heptafluoro-7,7-dimethyl-4,6-octanedione) europium (111)] was then added to the solution of the diester and the NMR spectrum redetermined.

The paramagnetic shifts produced in the different proton signals by varying concentrations of $Eu(fod)_3$ are given in Table 1 and these show that the shifts in the signals produced by the reagent decreased in the order vinyl H > βH > vinyl CH_3 > αH.

TABLE 1. PARAMAGNETIC SHIFTS FOR THE RESONANCES OF ASSIGNED PROTONS MEASURED FROM THE NMR SPECTRA OF THE DIMETHYL ESTER OF L-γ-ETHYLIDENEGLUTAMIC ACID

$Eu(fod)_3$ (mg/0·4 ml $CDCl_3$)	$\Delta\omega$ Hz Vinyl H	Vinyl CH_3	βH	αH
0	0	0	0	0
5	−10	−6	−8	−2
10	−15	−9	−13	−5
15	−27	−20	−25	−9
20	−39	−27	−35	−18
25	−51	−35	−48	−29
35	−84	−58	−75	−52
45	−103	−77	−79	−75
50*	−117	−77	−80	−73
60	−162	−83	−87	−81
75	−310	−107	−112	−90

* The shifts recorded for the methyl protons of the α and γ ester groups at a concentration of 50 mg $Eu(fod)_3$/0·4 ml $CDCl_3$ were −75 and −208 respectively.

Hinckley[10] has shown that the size of the paramagnetic shift is inversely proportional to the cube of the distance which separates the proton or protons affected from the coordination site. The shifts obtained in our experiments indicated that the Europium ion was coordinated to the carbonyl oxygen of γ-ester group, the effect of added $Eu(fod)_3$ on the signal given by the CH_3 of the γ-ester group being large and that on the signal given by the CH_3 of the γ-ester group small (footnote, Table 1). Shift differences between the other proton signals (Table 1) showed that the γ-ester group to which the europium ion was coordinated was closer to the vinyl H than to the vinyl CH_3 group, proving conclusively that the diester and therefore the original amino acid has a *cis* configuration about the double bond.

This finding was confirmed by measuring the distance between the coordination site (assumed to be identical with the carbonyl oxygen) and the various protons in molecular models of the *cis* and *trans* isomers (the most probable conformer being chosen in each case). The cubed reciprocal of each distance (Table 2) was then plotted against the appropriate

[10] C. C. HINCKLEY, *J. Am. Chem. Soc.* **91**, 5160 (1969).

TABLE 2. THE RELATIONSHIP BETWEEN OBSERVED PARAMAGNETIC SHIFTS AND CHELATION SITE—PROTON DISTANCES MEASURED FROM MOLECULAR MODELS

Protons	Δω, Hz	R(A°) *cis* Isomer	*trans* Isomer
Vinyl H	−117	2·5	4·5
Vinyl CH_3	−77	4·7	3·5
βH	−80	4·0	3·0
αH	−73	5·5	4·8

paramagnetic shift [produced by a concentration of 50 mg $Eu(fod)_3$/0·4 ml $CDCl_3$]: the values for the *cis* isomer showed a linear relationship whilst those of the *trans* isomer did not

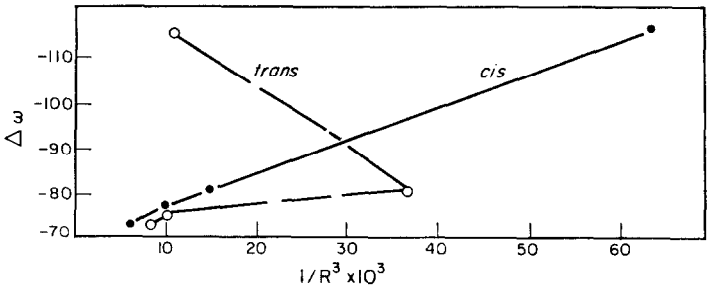
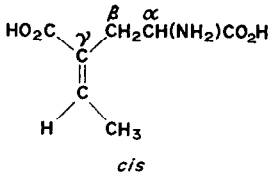
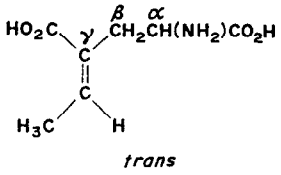


FIG. 1. PLOT OF Δω VS. $1/R^3$ SHOWING A LINEAR RELATIONSHIP FOR THE *cis* ISOMER. For a concentration of 50 mg $Eu(fod)_3$/0·4 ml $CDCl_3$ (see Table 1).

(Fig. 1). These findings provide unequivocal evidence that L-γ-ethylideneglutamic acid isolated from all three plant species has the *cis* configuration.



cis



trans

EXPERIMENTAL

Isolation of L-γ-*ethylideneglutamic acid.* Finely ground seed (170 g) from which the seed coats had been removed was soaked in CCl_4 (1 l.) for 10 hr to remove lipids and pigments. After filtration the air-dried seed was extracted with 75% EtOH (2 × 1 l.) at room temp. and with 50% EtOH (2 l.) at 60°. The combined extracts were taken to dryness under reduced pressure at 40° and the residue redissolved in H_2O (150 ml). The solution was applied to a column (120 × 4 cm) of strongly acidic resin (Amberlite CG 120, 100–200 mesh) in the H^+ form. The resin was then washed with H_2O (4 l.) and the amino acids displaced with 2 N NH_3 (3 l.). The solution of amino acids was taken to a syrup under reduced pressure in a rotary evaporator and finally to dryness (8 g crude amino acids) over P_2O_5. The mixture of amino acids was dissolved in water (20 ml) and applied to a column (90 × 4 cm) of a strongly basic ion-exchange resin (Amberlite CG-400, 100–200 mesh) in the acetate form. The basic and neutral amino acids were eluted from the column with H_2O (1870 ml) and the column treated successively with 0·1 M HOAc (900 ml), 0·2 M HOAc (900 ml) and 0·3 M

HOAc. After adding 490 ml of 0·3 M HOAc, L-γ-ethylideneglutamic acid appeared in the effluent and was eluted by itself in the following 500 ml (the other acidic amino acids were eluted later). The fractions containing γ-ethylideneglutamic acid were taken to dryness under reduced pressure and the last traces of acetic acid removed by redissolving the residue in water and evaporating to dryness × 4. The residue (500 mg) was finally recrystallized in colourless needles from a minimum volume of hot water. Yield 470 mg (0·28%). m.p. 198–201°; $[\alpha]_D^{25}$ +24·3° (*c*, 3·0; H_2O) $[\alpha]_D^{25}$ +41·2 (*c*, 1·5; 6 N HCl). The R_f values of the compound on paper [in *n*-BuOH–HOAc–H_2O (12:3:5) and phenol–H_2O (4:1 in the presence of NH_3)] and its ionic mobilities (at pH 1·9, 3·6, 4·4 and 6·5) were identical with those of authentic L-γ-ethylideneglutamic acid, as were the NMR and IR spectra.

The synthesis of the dimethyl ester of L-γ-*ethylideneglutamic acid.* To L-γ-ethylideneglutamic acid (100 mg) was added dry freshly distilled MeOH (15 ml). Dry HCl was bubbled through the solution at room temp. for 15 min. The temp. was then raised to 50° and HCl bubbled through for a further 30 min. After removing the MeOH in a rotary evaporator, the residue was treated with 5% Na_2CO_3 solution (15 ml) and extracted with $CHCl_3$ (40 ml). The extract was dried over anhydrous Na_2SO_4 for 2 hr and the $CHCl_3$ removed. The oily residue was distilled to give 26 mg of the dimethyl ester (b.p. 81–84° at 1–2 mm), NMR spectrum (in δppm, $CDCl_3$): A doublet, 1·88 (vinyl CH_3); a quartet, 7·15 (vinyl H); a triplet, 3·68 (αH); a doublet, 2·75 (β2H), a singlet, 3·75 (α ester CH_3) and a singlet, 3·79 (γ ester CH_3).

NMR spectrum in the presence of Eu(fod)$_3$. Solutions of the diester were prepared in $CDCl_3$ containing increasing amounts of Eu(fod)$_3$ (5–75 mg/0·4 ml $CDCl_3$) and the NMR spectrum of the compound redetermined at each concentration. The Eu(fod)$_3$ was supplied by Norell Chemical Company, Landing, NJ 07850, U.S.A. Spectra were recorded on a Varian A 60 spectrophotometer. The concentration of the diester was 1×10^{-4} M in 0·4 ml $CDCl_3$.

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