

ISOLATION AND SYNTHESIS OF GLUTAMINE AND GLUTARIMIDE DERIVATIVES FROM *CROTON HUMILIS*

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Abstract—Extract of *Croton humilis* L. were shown to contain N - [N - (2 - methylpropanoyl) - L - glutaminoyl] - 2 - phenylethylamine, N - [N - (2R - methylbutanoyl) - L - glutaminoyl] - 2 - phenylethylamine, 2 - [N - (2 - methylpropanoyl)] - N - phenylethylglutarimide and 2 - [N - (2R - methylbutanoyl)] - N - phenylethylglutarimide. Structural proof was based on acid degradation, spectral studies as well as the synthesis of N - [N - (2 - methylpropanoyl) - L - glutaminoyl] - 2 - phenylethylamine and 2 - [N - (2 - methylpropanoyl) - N - phenylethylglutarimide.

Previous studies reported the isolation of N - methyl - 2 - (p - hydroxyphenyl) ethylamine and N - methyl - 3 - (p - hydroxyphenyl) butylamine from *Croton humilis* L. (Euphorbiaceae),¹ and more recently we proposed structures for the 4:1 mixture of compounds, C₁₇H₂₅N₃O₃ (1) and C₁₈H₂₇N₃O₃ (2),² and the 1:1 mixture of C₁₇H₂₅N₃O₃ (3) and C₁₈H₂₇N₃O₃ (4) compounds.³ In the case of compounds 3 and 4 structural proposals were based on extensive INDOR studies⁴ as well as mass spectral investigations and acid degradative studies, which yielded glutamic acid, phenylethylamine, 2 - methylpropanoic and 2 - methylbutanoic acids. The latter compounds were identified as their 4 - bromophenacyl ester derivatives. Very recently, however, separation of this mixture of 3 and 4 has been achieved on preparative silica plates using methanol : chloroform (2:98). The C₁₈H₂₇N₃O₃ compound had m.p. 121–122°, [α]_D + 7.3°, and the UV, IR and NMR data were consistent with the glutarimide structure (4). ORD measurements gave a positive plain curve. High resolution mass spectrometric studies on the pure compound showed a fragmentation pattern which is summarised in Fig 1A. Acid degradation of this pure compound showed in conjunction with ORD data that the amino acid is L - (+) - glutamic acid.⁵ Structure 4 (without stereochemical definition) is of course identical with that proposed for the compound julocrotine isolated recently from *Julocroton mon-tevidensis* Klotzsch. (Euphorbiaceae).⁶ Julocrotine,

however, has m.p. 108–109°, [α]_D - 9°, and showed a negative plain curve in the ORD. Since acid degradation of julocrotine yielded L - (+) - glutamic acid and S - (+) - 2 - methylbutanoic acid⁶ and a sample kindly supplied by Professor Djerassi gave similar NMR and mass spectral data to our compound, it is clear that the C₁₈ - glutarimide derivative from *Croton humilis* contains R - (-) - 2 - methylbutanoic acid, L - (+) - glutamic acid and phenylethylamine.

The lower homolog, or C₁₇H₂₅N₃O₃ compound 3 was recently also obtained pure, and had m.p. 148–155°, [α]_D + 11° and gave a positive plain ORD curve. As in the case of compound 4, the IR showed an amide band at 3390 cm⁻¹ as well as the characteristic glutarimide adsorption at 1724 cm⁻¹. The NMR was consistent with structure 3 and electron initiated fragmentation as followed by high resolution measurements is summarised in Fig 1B. Acid degradation of this compound also showed the presence of L-(+)-glutamic acid.

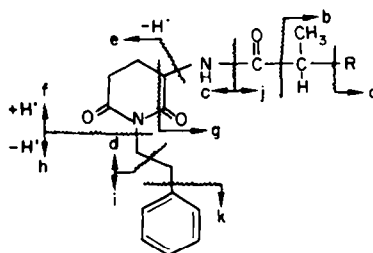


Fig 1. Fragmentation of glutarimides: 4, R = -CH₂CH₃ (A); 3, R = CH₃ (B).

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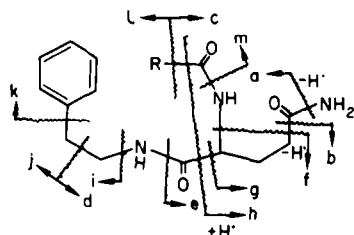


Fig. 2. Fragmentation of amide compounds 2, R = (CH₃CHCH₂CH₃); 1, R = CH(CH₃)₂ or CH(CH₃)CH₂CH₃;
1, R = CH(CH₃)₂.

Final confirmation of structure 3 was by synthesis.* Diethyl-(L)-glutamate⁷ was first converted to N-methylpropanoylglutamic acid diethyl ester. Base hydrolysis to the corresponding γ -ethyl, N-methylpropanoylglutamic acid α -(β -phenylethyl)-amide. Cyclisation to the glutarimide 3 was achieved with heat treatment at reduced pressure. This product was identical in all respects (m.p., m.m.p., IR, and $[\alpha]_D$) to the natural product.

In the case of the mixture of C₁₈H₂₇N₃O₃ and

C₁₇H₂₅N₃O₃ amides, no separation has been achieved, but structural assignments, namely structures 2 and 1 respectively could be made with confidence mainly on the basis of acid degradation to yield L-(+)-glutamic acid,⁵ phenylethylamine, 2-methylbutanoic, 2-methylpropanoic acids and ammonia. These results are supported by NMR data and extensive high resolution mass spectrometric studies. (Table and Fig 2).

Fragmentation *f* and *h* (Fig 2) preclude a γ -glutamyl linkage. ORD of the mixture gave a negative Cotton curve having a trough at 240 nm. This is in contrast to the ORD data reported for julocrotic acid A (5) and julocrotic acid B (6) which were obtained by degradation of julocrotine. These latter compounds were measured only down to 300 nm but both showed positive plain curves.⁶ They were shown to contain L-(+)-glutamic, S-(+)-2-methylbutanoic acids, and phenylethylamine. Since amides behave optically in an essentially similar fashion to carboxylic acids,⁸ it is evident that the C₁₈H₂₇N₃O₃ compound from *Croton humilis* is built up from L-(+)-glutamic, R-(-)-2-methylbutanoic acids and phenylethylamine, and is represented by structure 2.

The synthesis of compound 1, namely N-[N-(2-methylpropanoyl) - L - glutaminoyl] - 2 - phenylethylamine was achieved by treating a solution of γ -ethyl, N - methylpropanoyl glutamic acid α - (β -phenylethyl) amide with dry ammonia at 0° for 2 h.⁹

*Attempts to prepare compound 3 starting with N-t-BOC-L-glutamic acid γ -benzyl ester were unsuccessful. After condensation with phenylethylamine and removal of the BOC-group, acylation with isobutyryl chloride failed under several reaction conditions.

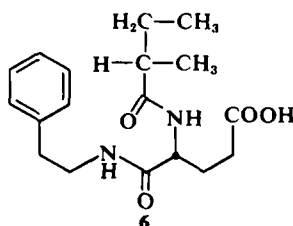
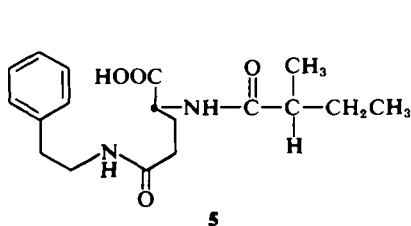
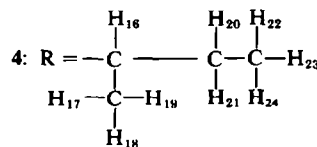
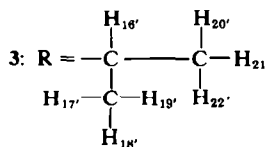
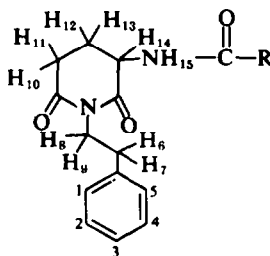
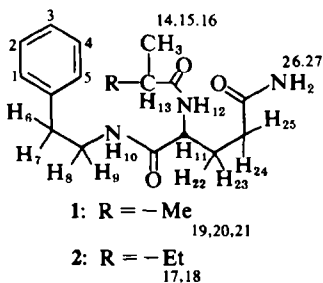


Table. High resolution mass spectral data on the mixture of amides (1 and 2)

Fragmentation (Fig. 2)	Obs.	% intensity	Elemental Comp.	Calc.	Pathway	Obs.	Calc.
*M ₁ ⁺	333.2044	10	C ₁₈ H ₂₇ N ₃ O ₃	333.2052			
M ₂ ⁺	319.1919	2.5	C ₁₇ H ₂₃ N ₃ O ₃	319.1896			
a ₁	316.1805	1	C ₁₈ H ₂₄ N ₂ O ₃	316.1787	333 → 316	299.7	299.7
a ₂	302.1612	0.25	C ₁₇ H ₂₂ N ₂ O ₃	302.1630	319 → 302	285.8	285.8
b ₁	289.1900	8	C ₁₇ H ₂₂ N ₂ O ₂	289.1916	333 → 289	250.9	250.9
c ₁	276.1342		C ₁₆ H ₁₈ N ₃ O ₃	276.1348			
b ₂	275.1757	2.5	C ₁₆ H ₂₃ N ₂ O ₂	275.1759	319 → 275	237.0	237.0
d ₁	242.1515	5	C ₁₁ H ₂₀ N ₃ O ₃	242.1505			
d ₂	228.1349	2	C ₁₀ H ₁₈ N ₃ O ₃	228.1348			
e ₁	213.1234	18	C ₁₀ H ₁₇ N ₂ O ₃	213.1239	333 → 213	136.2	136.2
e ₂	199.1082	4.5	C ₉ H ₁₃ N ₂ O ₃	199.1083	228 → 199	173.6	173.7
					319 → 199	124.0	124.1
f _{1,2}	188.1077	18	C ₁₂ H ₁₄ NO	188.1075	289 → 188	122.2	122.2
g ₁	185.1289	21	C ₉ H ₁₇ N ₂ O ₂	185.1290	333 → 185	102.7	102.8
					213 → 185	160.7	160.7
g ₂	171.1136	5	C ₈ H ₁₃ N ₂ O ₂	171.1134	319 → 171	91.65	91.65
h _{1,2}	129.0671	32	C ₅ H ₉ N ₂ O ₂	129.0664	213 → 129	78.1	78.1
	122.0976	12	C ₈ H ₁₂ N	122.0970	205 → 122	72.6	72.6
	105.0702	32	C ₈ H ₆	105.0704	122 → 105	90.4	90.4
i _{1,2}	104.0629	58	C ₈ H ₈	104.0626			
	101.0712	35	C ₈ H ₆ N ₂ O	101.0715	171 → 101	59.5	59.6
j _{1,2}	91.0541	31	C ₇ H ₇	91.0548			
	85.0645	25	C ₅ H ₇ O	85.0653			
	84.0447	60	C ₄ H ₆ NO	84.0449	101 → 84	69.8	69.8
	83.0615	32	C ₄ H ₇ N ₂	83.0609			
k _{1,2}	77.0386	5	C ₆ H ₅	77.0391			
l ₁	57.0693	100	C ₄ H ₆	57.0704			

Subscript 1 relates to C₁₈H₂₇N₃O₃ compound and subscript 2 to the C₁₇H₂₃N₃O₃ compound.

The product, m.p. 214–217°, gave the expected spectroscopic and analytical data.

S-(+)-2-Methylbutanoic acid occurs in julocrotine, and has also been reported as a phorbol ester (B₁-ester) from *Croton tiglium*¹⁰ and forms part of the structure of detoxin D isolated from *Streptomyces caespitosus* var. *detoxicus*.¹¹ To our knowledge the characterisation of R-(-)-2-methylbutanoic acid from a natural product has up to now not been reported, so the isolation of this enantiomer is a novel finding.

Both compounds 1 and 2 have been shown to contain a terminal amide group, and since ammonium hydroxide was used at one stage during the isolation process, there is the remote possibility that these compounds could have been produced by alkali hydrolysis of a structure containing an amidine grouping, in a manner analogous to that demonstrated for kikumycin A and B.¹²

Because of the known biological activity of several glutarimides, the C₁₈H₂₇N₃O₃ and C₁₇H₂₃N₃O₃ mixture was tested for antibacterial activity. These *in vitro* studies showed, however, that they were inactive against several commonly occurring pathogens and commensals.¹³

Pharmacological tests on several isolated tissue preparations were also performed with these compounds, but their low solubility in suitable solvents produced inconclusive results.¹⁴

EXPERIMENTAL

Isolation of glutarimides and other compounds from *Croton humilis*. In a typical extraction, 2 kg of dried and powdered plant material* (leaves and twigs) from Port Henderson, Jamaica, were slowly percolated with 2% tartaric acid (28 h) until the resulting extract gave no ppt with Mayer's reagent. This soln (15l) was concentrated to 1l. On standing in a refrigerator overnight, non-alkaloidal salts separated, and were filtered off. The filtrate was adjusted to pH8 with ammonium hydroxide and continuously extracted with chloroform by liquid-liquid extraction until the soln was negative to Mayer's reagent. Removal of the solvent yielded 10.1 g of a brown gummy material. Separation using 5% NaOH yielded 4.2 g of non-phenolic material and 3 g of phenols.¹

Addition of acetone to the non-phenolic fraction caused the precipitation of 77 mg of a white solid which was identified as a mixture of two amides,² C₁₈H₂₇N₃O₃ and C₁₇H₂₃N₃O₃, the examination of which is reported below. Separation of the rest of the material on PLC in MeOH:CHCl₃ (5:95 v/v) yielded two stable fractions. One of these consisted of a waxy compound, C₂₃H₃₂O₂, of undetermined structure; M⁺ 340.2374; calculated for C₂₃H₃₂O₂, 340.2401. (Found: C, 81.10; H, 9.30. C₂₃H₃₂O₂ Requires: C, 81.13; H, 9.47%. The other fraction yielded a

*Voucher No. 29,620 in the Herbarium, Botany Dept., U.W.I., Jamaica.

solid (85 mg), m.p. 132–138°, which by MS was shown to be a mixture of two related glutarimides, but which proved difficult to separate.³ This was finally achieved using MeOH:CHCl₃ (2:98 v/v). 820 mg of the mixture was placed on PLC and by arbitrarily dividing the plates, an "upper band" (260 mg) consisting of the C₁₈H₂₄N₂O₃ compound and a "lower band" (164 mg) of the C₁₇H₂₂N₂O₃ compound were obtained.

C₁₈H₂₄N₂O₃ Compound 4. m.p. 121–122° $\lambda_{\text{max}}^{\text{EtOH}}$ 210 nm (ϵ 18,540), $[\alpha]_{\text{D}} + 7.3^\circ \pm 2^\circ$ (CHCl₃); ORD (C, 0.59 in MeOH): $[\alpha]_{380} + 1.02^\circ$, $[\alpha]_{300} + 1.69^\circ$, $[\alpha]_{250} + 10.1^\circ$, $[\alpha]_{200} + 23.7^\circ$ and $[\alpha]_{230} + 32.8^\circ$; IR (CHCl₃), 3390 (amide) 2899, 1724 (glutarimide), 1681, 1493, 1342, 1156 and 1148 cm⁻¹; NMR (60 MHz, in CDCl₃, TMS reference): δ 7.27 (s, 5H, H-1 \rightarrow H-5), 6.37 (bs, 1H, H-15), 4.52 (m, 1H, H-14), 4.05 (m, 2H, H-9 and H-8), δ 2.60–2.97 (2H, H-6 and H-7), 2.75–1.4 (5H, H-10, H-11, H-12, H-13, H-17), 1.6 (2H, H-20 and H-21), 1.20 (d, J = 6 Hz, 3H, H-17, H-18 and H-19) and 0.90 (t, J = 7 Hz, 3H, H-22, H-23 and H-24). High resolution mass spectrometry*: $M^+ + 1$ 317.185 (16%) Calc for C₁₈H₂₄N₂O₃: 317.186; M^+ 316.180 (66%) C₁₈H₂₄N₂O₃ requires: 326.179: (a), \dagger 288.144 (2%), C₁₈H₂₀N₂O₃ requires: 288.147: (b) 259.105 (3%), C₁₈H₁₈N₂O₃ requires: 259.108: (c), 231.112 (5%), C₁₇H₂₂N₂O₃ requires: 231.113: (d), 225.125 (2%), C₁₇H₂₀N₂O₃ requires: 225.124: (e) 215.096 (7%), C₁₇H₁₈N₂O₃ requires: 215.095: (f), 212.116 (2%), C₁₆H₁₆N₂O₃ requires: 212.116: 196.096 (2%), C₁₆H₁₄N₂O₃ requires: 196.097: 184.083 (2%), C₁₆H₁₂N₂O₃ requires: 184.085: 168.104 (3%), C₁₆H₁₀N₂O₃ requires: 168.102: (g), 141.071 (2%), C₁₅H₁₀N₂O₃ requires: 141.078: 128.107 (6%), C₁₅H₈N₂O₃ requires: 128.107: (h), 104.063 (100%), C₈H₈ requires: 104.063: (i), 91.055 (7%), C₇H₈ requires: 91.055: (j), 85.066 (18%), C₇H₆ requires: 85.065: 84.046 (29%), C₆H₆NO requires: 84.045: (k) 77.035 (3%), C₆H₆ requires: 77.039: 57.030 (49%), C₅H₆O requires: 57.032.

Acid hydrolysis of the C₁₈H₂₄N₂O₃ compound to yield glutamic acid and phenylethylamine. Compound 4 (40 mg) was heated with 6N HCl and a minimum of MeOH 15 105° for 22 h. The degradation mixture was evaporated *in vacuo* and glutamic acid (15 mg) isolated by PLC on cellulose plates. Glutamic acid was identified by comparison with an authentic sample on cellulose in the solvent systems BuOH:HAc:H₂O (3:1:1) and (4:3:1), and identity confirmed by automatic amino acid analysis. ORD of this amino acid in 0.5M HCl showed a positive Cotton curve with trough at 224 nm, which is characteristic of L-(+)-glutamic acid.⁵ Phenylethylamine was also identified (IR, TLC) from the hydrolysis mixture.

Isolation and characterisation of 2-methylbutanoic acid and 2-methylpropanoic acid. The glutarimide mixture (60 mg) was hydrolysed as indicated above. A mixture of 4-bromophenylacetyl bromide derivatives of 2-methylpropionic and 2-methylbutanoic acids prepared from this reaction were separated on silica, and their identity confirmed by IR, TLC and NMR.

C₁₇H₂₂N₂O₃ Compound (3). m.p. 148–155°, $\lambda_{\text{max}}^{\text{EtOH}}$ 210 nm (ϵ 17,560), $[\alpha]_{\text{D}} + 11.6^\circ \pm 2^\circ$ (CHCl₃), ORD (c, 0.48 in MeOH): $[\alpha]_{380} + 16.8^\circ$, $[\alpha]_{300} + 16.4^\circ$, $[\alpha]_{250} + 16.0^\circ$, $[\alpha]_{200} + 12.5^\circ$, $[\alpha]_{230} + 10^\circ$, $[\alpha]_{230} + 3.0^\circ$; IR (CHCl₃), 3390 (amide), 2899, 1724 (glutarimide), 1681, 1493, 1339, 1156, 1148 cm⁻¹; NMR (60M Hz, in CDCl₃, TMS reference), showed signals at δ 7.27 (s, 5H, H-1, H-2, H-3, H-4, H-5), 6.37 (bs, 1H, H-15), 4.45 (m, 1H, H-14), 4.05 (m, 2H, H-8 and H-9), 2.62 to 2.97 (2H, H-6 and H-7), 2.75 to 1.4 (H-10,

H-11, H-12, H-13 and H-16'), 1.15 (d, J = 7.5 Hz, 6H, H-17', H-18', H-19', H-20', H-21', H-22'). Major fragments seen in a high resolution mass spectra are: M^+ 302.162 (9%). Calc for C₁₇H₂₂N₂O₃: 302.163; 232.117 (0.4%), C₁₇H₁₈N₂O₃ requires: 232.121; (e) \dagger , 215.097 (1.2%), C₁₇H₁₆N₂O₃ requires: 215.095; (h) 104.063 (86%), C₈H₈ requires: 104.063; (i) 91.054 (8%), C₇H₈ requires: 91.055; (j) 71.050 (6%), C₆H₆O requires: 71.050: 57.033 (4%), C₅H₆O requires: 57.034.

Acid hydrolysis of the C₁₇H₂₂N₂O₃ compound (3). The amide (20 mg) was hydrolysed with 6N HCl at 105° for 24 h. L-(+)-Glutamic acid was separated and determined as described for the C₁₈-compound. Phenylethylamine was also shown to be present.

Double resonance and INDOR studies on a 1:1 mixture of the glutarimides (3, 4). (100 MHz in CDCl₃ with a TMS lock). The mixture showed a singlet at δ 7.17 (H-1, H-2, H-3, H-4, H-5), a broad singlet at 6.3 (H-15), an 11-line multiplet at 4.43 (H-14; on irradiation of 1/2 height width of the 6.3 signal (5.5 mm) was reduced to 3.5 mm, and by double resonance was also shown to be coupled to signals at δ 1.5 and 2.5). A multiplet appeared at δ 4.0 (H-9, H-8; INDOR showed it coupled to signals at 2.65–2.86 (H-6, H-7). A group of signals occurred between 2.75 and 1.4 (H-10, H-11, H-12, H-13), while others were centred at 2.5 (H-16'), 2.2 (H-16). A doublet appeared at 1.13 (J = 6.5 Hz; H-17', H-18', H-19', H-20', H-21', H-22') and were further split, J = 0.5 Hz). A group of signals occurred at 1.6 and by INDOR were shown to be part of an Et group (H-20, H-21). A doublet was centred at 1.10 (J = 6.5 Hz; H-17, H-18, H-19) a triplet at 0.90 (J = 7 Hz and further split into 6 lines, J = 3 Hz) was assigned to H-22, H-23 and H-24.

Spectral examination of julocrotine from Julocroton montevidensis Klotzsch. NMR at 60M Hz in CDCl₃ showed signals at δ 7.22 (s, 5H, H-1 \rightarrow H-5), 6.26 (1H, H-15), 4.42 (m, 1H, H-14), 3.98 (m, H-8, H-9), 2.60–2.97 (m, 2H, H-6, H-7), 2.60–1.3 (5H, H-10 \rightarrow H-13 and H-17), 1.6 (2H, H-20, H-21), 1.17 (d, J = 6 Hz, 3H, H-17, H-18, H-19), 0.90 (t, J = 7 Hz, H-22, H-23, H-24). High resolution mass spectrometry: M^+ 317.186 (0.7%); Calc for C₁₈H₂₄N₂O₃: 317.186. The fragmentation pattern was identical to that of 4 except for differences in the relative abundance of the various ions.

Acid degradation of a mixture of the C₁₆H₁₈N₂O₃ and C₁₇H₂₂N₂O₃ amides. * (For isolation: please refer to K. L. Stuart and D. Y. Byfield.¹)

Experiment 1. ORD measurement on glutamic acid obtained by acid degradation of 50 mg of the mixture showed that L-(+)-glutamic acid was present.³ Phenylethylamine was also identified. The presence of 2-methylbutanoic and 2-methylpropanoic acid was proven by the preparation of 4-bromophenylacetyl bromide derivatives.

Experiment 2. 2.444 mg of the mixture was hydrolysed in a sealed tube at 105° for 24 h with 6N HCl, and automatic amino acid analysis on a 1/40 aliquot gave quantitative yields of glutamic acid, ammonia and phenylethylamine.

Spectral examination of a 4:1 mixture of C₁₈H₂₄N₂O₃ and C₁₇H₂₂N₂O₃ amides. The NMR in DMSO (100 MHz) showed the following features: δ 7.80 (m, ~ 2H, H-26, H-27), 7.20 (s, ~ 6H, H-1 \rightarrow H-5, H-12), 6.90 (t, ~ 1H, J = 2.6 Hz, H-10), 4.15 (m, ~ 1H, H-11), 3.25 (~ 2H, H-8, H-9), 2.65 (t, J = 6 Hz, ~ 2H, H-6, H-7), 1.7–2.3 (~ 5H, H-13, H-22 \rightarrow H-25), 1.3 (m, ~ 2H, H-17, H-18), 1.05 (d, J = 8 Hz ~ 3H, H-14, H-15, H-16), 0.08 (t, J = 8 Hz, ~ 3H, H-19, H-20, H-21); ORD (c, 0.47 in MeOH): $[\alpha]_{380}^{\text{DMSO}}$, $[\alpha]_{330} - 4.4^\circ$, $[\alpha]_{300} - 6.4^\circ$, $[\alpha]_{260} - 8.8^\circ$, $[\alpha]_{230} - 12.8^\circ$, $[\alpha]_{200} - 25.6^\circ$, $[\alpha]_{220} - 114^\circ$ and a trough at $[\alpha]_{240} = -163^\circ$.

* AEI MS-902 instrument.

\dagger Indicates fragmentation pathways (Fig. 1A).

\ddagger Mass spectral fragmentation Fig 1B.

Mass spectrometry was used to prove the presence of a mixture, by comparing the peak intensity of the parent ions m/e 333 and 319 and the fragments m/e 213 and 199. Voltages of 8, 10, 12, 16, 20 and 70 eV were used and a ratio 10:2.5 was consistently obtained. Table indicates the data obtained from a high resolution mass spectrometric study.

N-Methylpropanoylglutamic acid diethyl ester. To a soln of diethyl-(L)-glutamate (5.5 g; 27 mmole) prepared from recrystallised L-(+)-glutamic acid,⁷ was added 30 g of dry Dowex-1-X8 anion exchange resin (—OH form).

2-Methylpropanoyl chloride (4.4 g; 43 mmole) in 10 ml C_6H_6 was added dropwise to the magnetically stirred slurry. After approximately 12 h stirring, the slurry was filtered through sintered glass and the soln evaporated to dryness to give 2.5 g of a green oil. PLC of the oil on silica (R_f = 0.15) in $CHCl_3$ gave 2.1 g of product (30% yield); NMR ($CDCl_3$), δ 1.16 (dd, 6H, isopropyl C-Me), 1.25 (t, 3H, J = 7 Hz γ -OCH₂CH₃), 1.28 (t, 3H, J = 7 Hz, α -OCH₂CH₃), 2.18–2.56 (m, 5H, isopropyl C—H, and $2 \times$ —CH₂), 4.15 (q, J = 7 Hz, 2H, γ -OCH₂CH₃), 4.22 (q, J = 7 Hz, 2H, α -OCH₂CH₃), 4.7 (m, 1H, asym. C—H), 6.56 (broad d, 1H, —NH).

Methylpropanoylglutamic acid γ -ethyl ester. The above diethyl ester (1.9 g, 7 mmole) was partially hydrolysed in a manner similar to that for the preparation other γ -glutamic acid derivatives.¹⁵ The product (0.9 g) showed in the NMR ($CDCl_3$), signals at δ 1.18 (dd, 6H, isopropyl—C—CH₃), 1.21 (t, J = 7 Hz, 3H, γ -OCH₂CH₃), 2.1–2.7 (m, 4H, $2 \times$ —CH₂—), 4.16 (q, J = 7 Hz, 2H, γ -OCH₂CH₃) 4.55 (m, 1H, asym. —CH).

γ -Ethyl, N-methylpropanoylglutamic acid α -(β -phenylethyl)-amide. To a soln of the above γ -ethyl ester (900 mg; 3.67 mmole) and β -phenylethylamine (420 mg; 3.48 mmole) in 20 ml CH_2Cl_2 was added dropwise 900 mg of DCC (4.2 mmole) in 20 ml CH_2Cl_2 . After 30 min the soln became cloudy. The mixture was stirred in a stoppered flask for 36 h. Two drops of AcOH was added to the mixture and stirring continued for a final 1 h. 10 ml light petroleum was then added and the mixture filtered to remove the dicyclohexyl urea formed. The filtrate was evaporated to dryness to yield 1.5 g of a solid. PLC yielded 950 mg (yield 75%) of γ -ethyl, N-methylpropanoylglutamic acid α -(β -phenylethyl)amide, m.p. 94–97°; IR, ν_{max} ($CHCl_3$), 3370 (m), 3250 (m; —NH), 2900 (m; —CH), 1713 (s, ester), 1651 (s, amide), 1490 (s), 1440 (m), 1370 (m), 1220 (m). NMR ($CDCl_3$) δ 1.15 (dd, J = 11 Hz, 6H, isopropyl C-Me), 1.26 (t, J = 7 Hz, 3H, —CH₂CH₃), 2.0–2.60 (m, 4H, $2 \times$ —CH₂—), 2.83 (t, J = 7 Hz, 2H, benzylic—CH₂), 3.47 (t, J = 7 Hz, 2H, —CH₂), 4.15 (q, J = 7 Hz, 2H, —CH₂CH₃), 4.47 (m, 1H, asym. —CH), 6.70 (bd, 1H, —NH), 7.05 (bd, 1H, —NH), 7.25 (s, 5H, aromatic, H-1 \rightarrow H-5). High resolution mass spectrometry, M^+ 348.205 (11.8%), Calc for $C_{20}H_{25}N_2O_4$: 348.205. (Found: C, 65.66; H, 7.99; N, 8.11; O, 18.25. $C_{20}H_{25}N_2O_4$ requires: C, 65.49; H, 8.10; N, 8.04; O, 18.37%).

N-[N-(2-Methylpropanoyl)-L-glutaminoyl]-2-phenylethylamine (1). Dry ammonia was bubbled through a soln of γ -ethyl, N-methylpropanoylglutamic acid α -(β -phenylethyl) amide, (400 mg; 1.16 mmole), in 6 ml abs MeOH at 0° for 2 h.⁹ The residue on evaporation was crystallised from EtOH: C_6H_6 (1:9 v/v) to give 100 mg of 1, m.p. 214–217°; IR ($CHCl_3$), 3320 (m) (NH), 3224 (s, NH), 1658 (s) (CO of amide), 1630 (s, CO of amide), 1615 (s, amide), 1435 (m), 1430 (w), 1282 (w), 1232 (w); NMR (DMSO) δ 1.01 (overlapping doublet J = 11 Hz, 6H, iso-

propyl C—Me), 1.60–2.10 (m, 5H, H-22 \rightarrow H-25 + —CH₂ of isopropyl) 2.5–2.9 (t, J = 7 Hz, 2H, H-6, H-7), 3.0–3.3 (m, 2H, H-8, H-9), 4.0–4.3 (m, 1H, H-11), 6.90 (1H, H-10), 7.25 (s, 6H, H-1 \rightarrow H-5 and H-12), 7.80 (m, 2H, H-26, H-27). High resolution mass spectrometry showed ions which could be identified as those observed in the amide mixture (Table). M^+ 319.1883 calc for $C_{17}H_{23}N_3O_3$: 319.1895; (a_2) 302.165, $C_{17}H_{23}N_3O_3$ requires: 302.163; (b_2) 275.175, $C_{16}H_{23}N_3O_3$ requires: 275.176; (d_2) 228.130, $C_{16}H_{18}N_3O_3$ requires: 228.135; (e_2) 199.109, $C_8H_{13}N_3O_3$ requires: 199.108; (f_2) 188.108, $C_{17}H_{14}NO$ requires: 188.108; (g_2) 171.111, $C_8H_{13}N_3O_2$ requires: 171.113; (h_2) 129.066, $C_8H_8N_2O_2$ requires: 129.066; (i_2) 104.062, C_8H_8 requires: 104.063; (j_2) 91.056, C_7H_7 requires: 91.055; (k) 77.037; C_6H_6 requires: 77.039; (m), 71.048, C_6H_6O requires: 71.049. (Found: C, 63.66; H, 7.90; N, 13.20; O, 15.17. $C_{17}H_{23}N_3O_3$ requires: C, 63.92; H, 7.89; N, 13.16; O, 15.03%).

2-[N-(2-Methylpropanoyl)-N-phenylethylglutarimide (3). Treatment of γ -ethyl, N-methylpropanoylglutamic acid α -(β -phenylethyl) amide (35 mg) at 250° for 30 min at 30 mm Hg yielded after separation on PLC a compound (20 mg) which gave identical NMR and IR spectra to the natural product (3). Further confirmation came from m.p. and mixed m.p. 148–155°, the optical activity $[\alpha]_D + 11^\circ \pm 2^\circ$ (c, 1.4 $CHCl_3$) and TLC behaviour in several systems. (Found: C, 65.95; H, 7.28; N, 8.77. Calc for $C_{17}H_{23}N_3O_3 \cdot \frac{1}{2}H_2O$: C, 65.61; H, 7.44; N, 8.99%).

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