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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, $C_{17}H_{25}N_3O_3$ (**1**) and $C_{18}H_{27}N_3O_3$ (**2**),[2] and the 1:1 mixture of $C_{17}H_{22}N_2O_3$ (**3**) and $C_{18}H_{24}N_2O_3$ (**4**) compounds.[3] In the case of compounds **3** and **4** structural proposals were based on extensive INDOR studies[4] 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 $C_{18}H_{24}N_2O_3$ compound had m.p. 121–122°, $[\alpha]_D + 7{\cdot}3°$, 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 montevidensis* Klotzsch. (Euphorbiaceae).[6] Julocrotine, however, has m.p. 108–109°, $[\alpha]_D - 9°$, and showed a negative plain curve in the ORD. Since acid degradation of julocrotine yielded L - (+) - glutamic acid and *S* - (+) - 2 - methylbutanoic acid[6] and a sample kindly supplied by Professor Djerassi gave similar NMR and mass spectral data to our compound, it is clear that the C_{18} - glutarimide derivative from *Croton humilis* contains *R* - (−) - 2 - methylbutanoic acid, L - (+) - glutamic acid and phenylethylamine.

The lower homolog, or $C_{17}H_{22}N_2O_3$ compound **3** was recently also obtained pure, and had m.p. 148–155°, $[\alpha]_D + 11°$ 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.

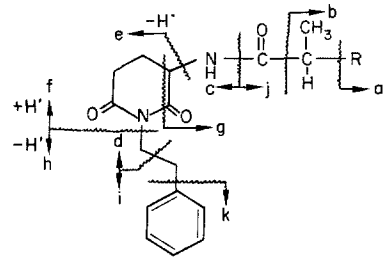

Fig 1. Fragmentation of glutarimides: **4**, R = $-CH_2CH_3$ (A); **3**, R = CH_3 (B).

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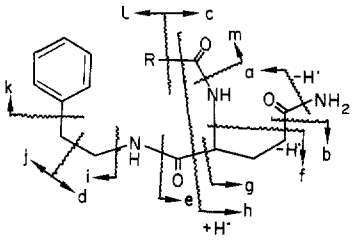


Fig 2. Fragmentation of amide compounds **2**, R = $(CH_3CHCH_2CH_3$; **1**, R = $CH(CH_3)_2$ or $CH(CH_3)CH_2CH_3$; **1**, R = $CH(CH_3)_2$.

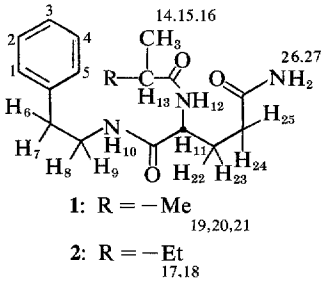
Final confirmation of structure **3** was by synthesis.* Diethyl-(L)-glutamate[7] was first converted to N-methylpropanoylglutamic acid diethyl ester. Base hydrolysis to the corresponding γ-ethyl ester was followed by conversion to γ-ethyl, N-methylpropanoylglutamic acid α-(β-phenylethyl)-amide. Cyclisation 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 $C_{18}H_{27}N_3O_3$ and $C_{17}H_{25}N_3O_3$ 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 mn. 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 $C_{18}H_{27}N_3O_3$ compound from *Croton humilis* is built up from L-(+)-glutamic, *R*-(−)-2-methylbutanoic acids and phenylethylamine, and is represented by structure **2**.

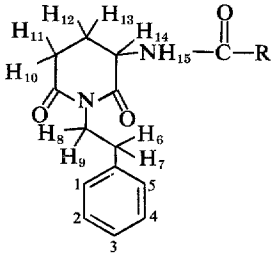
The synthesis of compound **1**, namely N-[N-(2-methylpropanoyl)-L-glutaminoyl]-2-phenylethylamine was achieved by treating a solution of γ-ethyl, N-methylpropanoyl glutamic acid α-(β-phenylethyl) amide with dry ammonia at 0° for 2 h.[9]

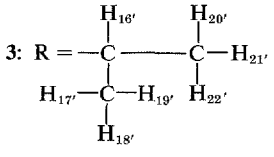
*Attempts to prepare compound **3** starting with N-t-BOC-L-glutamic acid γ-benzyl ester were unsuccessful. After condensation with phenylethylamine and removal of the BOC-group, acylation with isobutyryl chloride failed under several reaction conditions.

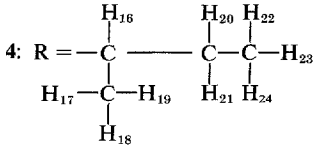


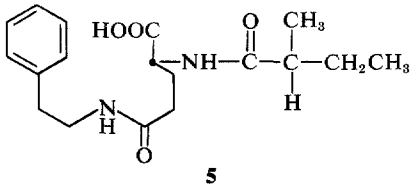
1: R = −Me (19,20,21)

2: R = −Et (17,18)









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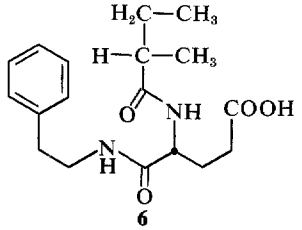

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Table. High resolution mass spectral data on the mixture of amides (**1** and **2**)

Fragmentation (Fig. 2)	Obs.	% intensity	Elemental Comp.	Calc.	Pathway	Obs.	Calc.
$*M_1^{\cdot+}$	333·2044	10	$C_{18}H_{27}N_3O_3$	333·2052			
$M_2^{\cdot+}$	319·1919	2·5	$C_{17}H_{25}N_3O_3$	319·1896			
a_1	316·1805	1	$C_{18}H_{24}N_2O_3$	316·1787	333→316	299·7	299·7
a_2	302·1612	0·25	$C_{17}H_{22}N_2O_3$	302·1630	319→302	285·8	285·8
b_1	289·1900	8	$C_{17}H_{25}N_2O_2$	289·1916	333→289	250·9	250·9
c_1	276·1342		$C_{14}H_{18}N_3O_3$	276·1348			
b_2	275·1757	2·5	$C_{16}H_{23}N_2O_2$	275·1759	319→275	237·0	237·0
d_1	242·1515	5	$C_{11}H_{20}N_3O_3$	242·1505			
d_2	228·1349	2	$C_{10}H_{18}N_3O_3$	228·1348			
e_1	213·1234	18	$C_{10}H_{17}N_2O_3$	213·1239	333→213	136·2	136·2
e_2	199·1082	4·5	$C_9H_{15}N_2O_3$	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	$C_9H_{17}N_2O_2$	185·1290	333→185	102·7	102·8
					213→185	160·7	160·7
g_2	171·1136	5	$C_8H_{15}N_2O_2$	171·1134	319→171	91·65	91·65
$h_{1,2}$	129·0671	32	$C_5H_9N_2O_2$	129·0664	213→129	78·1	78·1
	122·0976	12	$C_8H_{12}N$	122·0970	205→122	72·6	72·6
	105·0702	32	C_8H_9	105·0704	122→105	90·4	90·4
$i_{1,2}$	104·0629	58	C_8H_8	104·0626			
	101·0712	35	$C_4H_9N_2O$	101·0715	171→101	59·5	59·6
$j_{1,2}$	91·0541	31	C_7H_7	91·0548			
	85·0645	25	C_5H_7O	85·0653			
	84·0447	60	C_4H_6NO	84·0449	101→84	69·8	69·8
	83·0615	32	$C_4H_7N_2$	83·0609			
$k_{1,2}$	77·0386	5	C_6H_5	77·0391			
l_1	57·0693	100	C_4H_9	57·0704			

Subscript 1 relates to $C_{18}H_{27}N_3O_3$ compound and subscript 2 to the $C_{17}H_{25}N_3O_3$ 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 (B_1-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 $C_{18}H_{24}N_2O_3$ and $C_{17}H_{22}N_2O_3$ 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] $C_{18}H_{27}N_3O_3$ and $C_{17}H_{25}N_3O_3$, the examination of which is reported below. Separation of the rest of the material on PLC in MeOH:$CHCl_3$ (5:95 v/v) yielded two stable fractions. One of these consisted of a waxy compound, $C_{23}H_{32}O_2$, of undetermined structure; M^+ 340·2374; calculated for $C_{23}H_{32}O_2$, 340·2401. (Found: C, 81·10; H, 9·30. $C_{23}H_{32}O_2$ 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:$CHCl_3$ (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 $C_{18}H_{24}N_2O_3$ compound and a "lower band" (164 mg) of the $C_{17}H_{22}N_2O_3$ compound were obtained.

$C_{18}H_{24}N_2O_3$ *Compound* **4**. m.p. 121–122° λ_{max}^{EtOH} 210 nm (ϵ 18,540), $[\alpha]_D + 7{\cdot}3° \pm 2°$ ($CHCl_3$); ORD (*C*, 0·59 in MeOH): $[\alpha]_{589} + 1{\cdot}02°$, $[\alpha]_{500} + 1{\cdot}69°$, $[\alpha]_{350} + 10{\cdot}1°$, $[\alpha]_{300} + 23{\cdot}7°$ and $[\alpha]_{250} + 32{\cdot}8°$; IR ($CHCl_3$), 3390 (amide) 2899, 1724 (glutarimide), 1681, 1493, 1342, 1156 and 1148 cm^{-1}; NMR (60 MHz, in $CDCl_3$, TMS reference): δ 7·27 (s, 5H, H-1→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 = 6Hz, 3H, H-17, H-18 and H-19) and 0·90 (t, J = 7Hz, 3H, H-22, H-23 and H-24). High resolution mass spectrometry*: $M^+ + 1$ 317·185 (16%) Calc for $C_{18}H_{25}N_2O_3$: 317·186: M^+ 316·180 (66%) $C_{18}H_{24}N_2O_3$ requires: 326·179: (a),† 288·144 (2%), $C_{16}H_{20}N_2O_3$ requires: 288·147: (b) 259·105 (3%), $C_{14}H_{15}N_2O_3$ requires: 259·108: (c), 231·112 (5%), $C_{13}H_{17}N_2O_2$ requires: 231·113; (d), 225·125 (2%), $C_{11}H_{17}N_2O_3$ requires: 225·124; (e) 215·096 (7%), $C_{13}H_{13}NO_2$ requires: 215·095; (f), 212·116 (2%), $C_{10}H_{16}N_2O_3$ requires: 212·116; 196·096 (2%), $C_{10}H_{14}NO_3$ requires: 196·097; 184·083 (2%), $C_8H_{12}N_2O_3$ requires: 184·085; 168·104 (3%), $C_9H_{14}NO_2$ requires: 168·102; (g), 141·071 (2%), $C_7H_{11}NO_2$ requires: 141·078: 128·107 (6%), $C_7H_{14}NO$ requires: 128·107; (h), 104·063 (100%), C_8H_8 requires: 104·063: (i), 91·055 (7%), C_7H_7 requires: 91·055; (j), 85·066 (18%), C_5H_9 requires: 85·065:84·046 (29%), C_4H_6NO requires: 84·045; (k) 77·035 (3%), C_6H_5 requires: 77·039:57·030 (49%), C_3H_5O requires: 57·032.

Acid hydrolysis of the $C_{18}H_{24}N_2O_3$ *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:H_2O (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-bromophenylacylbromide 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.

$C_{17}H_{22}N_2O_3$ *Compound* **(3)**, m.p. 148–155°, λ_{max}^{EtOH} 210 nm (ϵ 17,560), $[\alpha]_D + 11{\cdot}6° \pm 2°$ ($CHCl_3$), ORD (*c*, 0·48 in MeOH): $[\alpha]_{589} + 16{\cdot}8°$, $[\alpha]_{500} + 16{\cdot}4°$, $[\alpha]_{400} + 16{\cdot}0°$, $[\alpha]_{350} + 12{\cdot}5°$, $[\alpha]_{300} + 10°$, $[\alpha]_{250} + 3{\cdot}0°$; IR ($CHCl_3$), 3390 (amide), 2899, 1724 (glutarimide), 1681, 1493, 1339, 1156, 1148·cm^{-1}; NMR (60M Hz, in $CDCl_3$, 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 $C_{17}H_{22}N_2O_3$: 302·163; 232·117 (0·4%), $C_{13}H_{16}N_2O_2$ requires: 232·121; (e)‡, 215·097 (1·2%), $C_{13}H_{13}NO_2$ requires: 215·095; (h) 104·063 (86%), C_8H_8 requires: 104·063; (i) 91·054 (8%), C_7H_7 requires: 91·055; (j) 71·050 (6%), C_4H_7O requires: 71·050: 57·033 (4%), C_3H_5O requires: 57·034.

Acid hydrolysis of the $C_{17}H_{24}N_2O_3$ *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 C_{18}-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 $CDCl_3$ 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 $CDCl_3$ showed signals at δ 7·22 (s, 5H, H-1→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→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 $C_{18}H_{25}N_2O_3$: 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 $C_{18}H_{27}N_3O_3$ *and* $C_{17}H_{25}N_3O_3$ *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.[5] Phenylethylamine was also identified. The presence of 2-methylbutanoic and 2-methylpropanoic acid was proven by the preparation of 4-bromophenacylbromide 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* $C_{18}H_{27}N_3O_3$ *and* $C_{17}H_{25}N_3O_3$ *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→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→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]_{589}$0°, $[\alpha]_{550}$–4·4°, $[\alpha]_{500}$–6·4°, $[\alpha]_{400}$–8·8°, $[\alpha]_{350}$–12·8°, $[\alpha]_{300}$–25·6°, $[\alpha]_{250}$–114° and a trough at $[\alpha]_{240} = -163°$.

*AEI MS-902 instrument.
†Indicates fragmentation pathways (Fig. 1A).
‡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-$OCH_2C\underline{H}_3$), 1·28 (t, 3H, J = 7 Hz, α-$OCH_2C\underline{H}_3$), 2·18–2·56 (m, 5H, isopropyl C—H, and 2× —$C\underline{H}_2$), 4·15 (q, J = 7 Hz, 2H, γ-$OC\underline{H}_2CH_3$), 4·22 (q, J = 7 Hz, 2H, α-$OC\underline{H}_2CH_3$), 4·7 (m, 1H, asymm. 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—$C\underline{H}_3$), 1·21 (t, J = 7 Hz, 3H, γ-$OCH_2C\underline{H}_3$), 2·1–2·7 (m, 4H, 2×—$C\underline{H}_2$—), 4·16 (q, J = 7 Hz, 2H, γ—$OC\underline{H}_2CH_3$) 4·55 (m, 1H, asymm. —CH).

γ-Ethyl, N-methylpropanoylglutamic acid α-(β-phenyl-ethyl)-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} ($CHCl_2$), 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, —$CH_2C\underline{H}_3$), 2·0–2·60 (m, 4H, 2×—$C\underline{H}_2$—), 2·83 (t, J = 7 Hz, 2H, benzyl-$C\underline{H}_2$), 3·47 (t, J = 7 Hz, 2H, —$C\underline{H}_2$), 4·15 (q, J = 7 Hz, 2H, —$C\underline{H}_2CH_3$), 4·47 (m, 1H, asymm. —CH), 6·70 (bd, 1H, —NH), 7·05 (bd, 1H, —NH), 7·25 (s, 5H, aromatic, H-1→H-5). High resolution mass spectrometry, M^+ 348·205 (11·8%), Calc for $C_{19}H_{28}N_2O_4$: 348·205. (Found: C, 65·66; H, 7·99; N, 8·11; O, 18·25. $C_{19}H_{28}N_2O_4$ 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, isopropyl C—Me), 1·60–2·10 (m, 5H, H-22→H-25 + —CH 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→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 $C_{17}H_{25}N_3O_3$: 319·1895; (a_2), 302·165, $C_{17}H_{22}N_2O_3$ requires: 302·163; (b_2) 275·175, $C_{16}H_{23}N_2O_3$ requires: 275·176; (d_2) 228·130, $C_{10}H_{18}N_3O_3$ requires: 228·135; (e_2) 199·109, $C_9H_{15}N_2O_3$ requires: 199·108; (f_2) 188·108, $C_{12}H_{14}NO$ requires: 188·108; (g_2) 171·111, $C_8H_{15}N_2O_2$ requires: 171·113; (h_2) 129·066, $C_5H_9N_2O_2$ 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_4 requires: 77·039; (m), 71·048, C_4H_7O requires: 71·049. (Found: C, 63·66; H, 7·90; N, 13·20; O, 15·17. $C_{17}H_{25}N_3O_3$ requires: C, 63·92; H, 7·89; H, 13·16; O, 15·03%).

2 - [N - (2 - *Methylpropanoyl*] - N - *phenylethyl-glutarimide* (**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 $[\alpha]_D + 11° \pm 2°$ (*c*, 1·4 $CHCl_3$) and TLC behaviour in several systems. (Found: C, 65·95; H, 7·28; N, 8·77. Calc for $C_{17}H_{22}N_2O_3 \cdot \frac{1}{2}H_2O$: C, 65·61; H, 7·44; N, 8·99%).

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REFERENCES

[1]K. L. Stuart and D. Y. Byfield, *Photochemistry* **10**, 460 (1971)
[2]J. P. Kutney, F. K. Klein, G. Knowles and K. L. Stuart, *Tetrahedron Letters* 3263 (1971)
[3]J. P. Kutney, F. K. Klein, G. Eigendorf, D. McNeill and K. L. Stuart, *Ibid.* 4973 (1971); *ibid* 250 (1972)
[4]R. Burton, L. D. Hall and P. R. Steiner, *Canad. J. Chem.* **48**, 2679 (1970) and ref cited
[5]W. Klyne and P. M. Scopes, *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry*, p. 200 (1967)
[6]T. Nakano, C. Djerassi, R. A. Corral and O. O. Orazi, *J. Org. Chem.* **26**, 1184 (1961)
[7]Z. Pravda, *Coll. Czech. Chem. Comm.* **24**, 2083 (1959)
[8]A. Moscowitz, A. Rosenberg and A. E. Hansen, *J. Am. Chem. Soc.* **87**, 1813 (1965)
[9a]M. Bodanszky and V. DuVigneaud, *Ibid.* **81**, 5688 (1959); [b]S. S. Wang, *Ibid.* **95**, 1329 (1973)
[10]E. Hecker, *Methods in Cancer Research* **6**, 439 (1971)
[11]K. Kakinuma, N. Otake and H. Yonehara, *Tetrahedron Letters* 2509 (1972)
[12]T. Takaishi, Y. Sugawara and M. Suzaki, *Ibid.* 1873 (1972)
[13]O. B. O. James and L. S. Grant, Unpublished observations
[14]V. Calver and P. C. Feng, Unpublished results
[15]W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.* 3239 (1950)