Tetrahedron Letters No. 35, pp.3263 - 3266, 1971. Pergamon Press. Printed in Great Britain.

ALKALOIDS FROM *CROTON* SPECIES. XI[1]. PEPTIDYL COMPOUNDS FROM *C. HUMILIS* L.

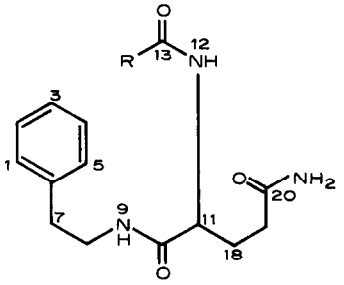
J.P. Kutney, F.K. Klein and G. Knowles
Department of Chemistry, University of British Columbia
Vancouver 8, Canada

and

K.L. Stuart
Chemistry Department, University of the West Indies
Kingston 7, Jamaica

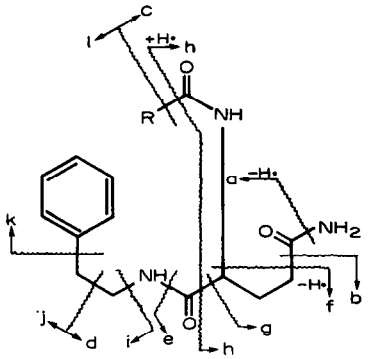
(Received in USA 28 May 1971; received in UK for publication 26 July 1971)

C. humilis L. (Euphorbiaceae), a West Indian shrub, has been shown to contain 2-methyl-2-butenoic acid[2], N-methyl-2-(p-hydroxyphenyl) ethylamine (N-methyltyramine), N-methyl-3-(p-hydroxyphenyl) butylamine (N-methylhomotyramine) and two other compounds, $C_{18}H_{27}N_3O_3$ and $C_{35}H_{51}NO_7$ of unknown structures.[3] We have reexamined the C_{18} compound and established that it consisted of a 4:1 mixture of the two compounds, $C_{18}H_{27}N_3O_3$ and $C_{17}H_{25}N_3O_3$. Spectroscopic and degradative evidence identified these compounds as N-[N-(2-methylbutanoyl)glutaminoyl]-2-phenylethylamine (I) and N-[N-(2-methylpropionoyl)glutaminoyl]-2-phenylethylamine (II). The β-glutamyl linkage of I and II is unique among a series of naturally-occurring γ-glutamyl oligopeptides.[4] However the phenylethylamide moiety more closely relates to similar structural features of peptide alkaloids.[5-7]



I, R = —CH(CH$_3$)—CH$_2$CH$_3$

II, R = —CH(CH$_3$)—CH$_3$



III

Interpretation of high resolution mass spectral data, in which all ions with relative abundances exceeding 4% were checked and all metastables assigned, fully accorded with the proposed structures (Table I). The postulated fragmentation processes are shown in III. Ions f and h preclude a γ-glutamyl linkage.

Table I. Mass Spectral Data on Peptidyl Compounds I and III.[a]

Ion	$C_{18}H_{27}N_3O_3$ (R = $-CH(CH_3)CH_2CH_3$)			$C_{17}H_{25}N_3O_3$ (R = $-CH(CH_3)_2$)		
	Found(m/e)	Formula	Metastable	Found(m/e)	Formula	Metastable
$M^{\cdot+}$	333.204	$C_{18}H_{27}N_3O_3$		319.191	$C_{17}H_{25}N_3O_3$	
a	316.180	$C_{18}H_{24}N_2O_3$	299.7	302.161	$C_{17}H_{22}N_2O_3$	285.8
b	289.189	$C_{17}H_{25}N_2O_2$	250.9	275.175	$C_{16}H_{23}N_2O_2$	237.0
c	276.134	$C_{14}H_{18}N_3O_3$		---		
d	242.151	$C_{11}H_{20}N_3O_3$		228.134	$C_{10}H_{18}N_3O_3$	
e	213.123	$C_{10}H_{17}N_2O_3$	136.2	199.108	$C_9H_{15}N_2O_3$	124.0
f	188.107	$C_{12}H_{14}NO$	122.2		$C_{12}H_{14}NO$	
g	185.128	$C_9H_{17}N_2O_2$	102.7	171.113	$C_8H_{15}N_2O_2$	91.6
h	129.067	$C_5H_9N_2O_2$	78.1		$C_5H_9N_2O_2$	
i	104.062	C_8H_8			C_8H_8	
j	91.054	C_7H_7			C_7H_7	
k	77	C_6H_5			C_6H_5	
l	57.069	C_4H_9				

[a] The data was obtained on an AEI MS-902 mass spectrometer at 70eV.

Because of the close structural similarity of the two homologs and the predominance of the C_{18} compound (I), the nmr spectrum could be completely analyzed and interpreted with confidence (Table II).

Table II. Nmr Spectral Data on Peptidyl Compound I.[a]

δ(p.p.m.)	Multiplicity	Integration	Assignment
0.80	t(J=8 Hz)	3H	16
1.05	d(J=8 Hz)	3	17
1.3	m	2	15
1.7-2.3	unresolved	5	14,18,19
2.65	t(J=6 Hz)	2	7
3.25	unresolved	2	8
4.15	m	1	11
6.90	t(J=2.6 Hz)	1	9
7.20	s	6	1-5,12
7.80	m	2	21

[a] The data was obtained on a Varian HA100 spectrometer in dimethylsulfoxide solution.

Hydrolysis of the mixture with 6N hydrochloric acid at 105° for 24 hours was shown by automatic amino acid analysis to yield one equivalent each of glutamic acid and ammonia. Glutamic acid and 2-phenylethylamine were confirmed by preparative tlc isolation and comparison with authentic samples; mass spectrometry of one fraction of the hydrolysate was also diagnostic for glutamic acid hydrochloride and ammonium chloride. However, insufficient quantities of the original mixture available precluded firm characterization of 2-methylbutanoic acid and 2-methylpropionic acids or the assignment of configuration at the C-11 and C-14 asymmetric centers.

Since C. humilis contains 2-methyl-2-butenoic acid as well as phenylalanine and glutamic acid,[8] it seems reasonable to speculate that the biogenetic pathway to the C_{18} homolog involves all these three compounds.

Although Croton species are known sources of proaporphine and morphinandienone alkaloids, no peptide alkaloids have yet been reported. The possible role of these peptidyl compounds in the plant is being evaluated.

Acknowledgment. Financial aid from the National Research Council of Canada is gratefully acknowledged. One of us (K.L. Stuart) wishes to acknowledge the assistance of a CIDA-NRC Research Associateship during the tenure of this work.

REFERENCES

1. Part X. K.L. Stuart, D. Byfield, C. Chambers and G.E.M. Husbands, J. Chem. Soc. (C), 1228 (1970).
2. N.R. Farnsworth, R.N. Blomster, W.M. Messmer, J.C. King, G.J. Persinos and J.D. Wilkes, Lloydia, 32, 1 (1969).
3. K.L. Stuart and D.Y. Byfield, Phytochemistry, 10, 460 (1971).
4. S.G. Waley, Adv. Protein Chem., 21, 1 (1966).
5. R. Tschesche, L. Behrendt and H.W. Fehlhaber, Chem. Ber., 102, 50 (1969).
6. M. Pais, J. Marchand, G. Ratle and F.X. Jarreau, Bull. Soc. Chim. France, 2979 (1968).
7. F.K. Klein and H. Rapoport, J. Amer. Chem. Soc., 90, 3576 (1968).
8. K.L. Stuart and D. McNeill, unpublished results.