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# The Synthesis and Structure of a Cyclobutane Analogue of Glutamic Acid with an Acetic Acid Side Chain

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*Abstract*

A synthetic route involving a hydantoin derivative of bicyclo[3.2.0]hept-2-ene has been investigated for the preparation of neurotransmitter analogues containing an additional acetic acid substituent on the cyclobutane ring of the potent NMDA receptor agonist *trans*-1-aminocyclobutane-1,3-dicarboxylic acid. X-Ray analysis showed that the major cyclobutane amino acid produced had the 2-acetic acid and 3-carboxylic acid substituents in the *trans* orientation as a result of epimerization during hydantoin hydrolysis.

## Introduction

The acidic amino acid L-glutamic acid acts as the major excitatory amino acid neurotransmitter in the mammalian central nervous system.[1,2] L-Glutamic acid activates a number of different receptors, and these receptors have been divided into a number of subtypes which directly activate cation channels in the postsynaptic membrane, as well as a metabotropic receptor subtype which modifies membrane potential through other mechanisms.[1,2] The main subtypes are classified by selective agonists that activate them into NMDA receptors which are activated by *N*-methyl-D-aspartic acid, and non-NMDA receptors which are activated by α-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid (AMPA) or kainic acid (1). Antagonists of varying degrees of selectivity have been developed for the different subtypes, but the NMDA receptor is the best understood in terms of structural requirements for agonists and antagonists.[3]

However, the alterations in agonist structure needed to change an NMDA agonist into a non-NMDA agonist are not well understood although there are some examples indicating that the receptors may be closely related. For example, the cyclopropane analogues (2) have been reported to act as a selective non-NMDA agonist when the methoxymethyl substituent is *trans* to the other substituents on the cyclopropane ring [(6*R*)-MOM-L-CCG-IV], while the *cis* isomer [(6*S*)-MOM-L-CCG-IV] interacts with NMDA receptors.[4] In this paper we report

[1] Rang, H. P., and Dale, M. M., 'Pharmacology' 2nd Edn, p. 593 (Churchill Livingstone: Edinburgh 1991).
[2] Watkins, J. C., Krogsgaard-Larsen, P., and Honoré, T., *Trends Pharmacol. Sci.*, 1990, **11**, 1175.
[3] Hansen, J. J., and Krogsgaard-Larsen, P., *Med. Res. Rev.*, 1990, **10**, 55.

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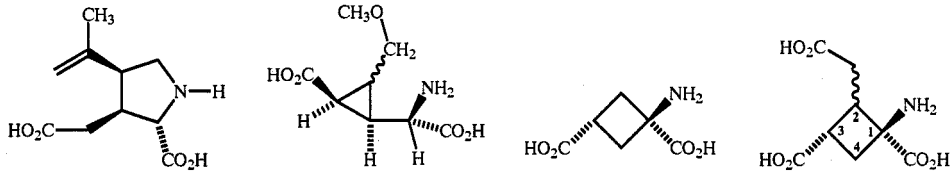
the incorporation of an extra functional group onto the potent NMDA receptor agonist *trans*-1-aminocyclobutane-1,3-dicarboxylic acid (3)[5] in order to probe for an extra binding site at excitatory amino acid receptors.




(1) (2) (3) (4)

For the kainic acid analogues, it has been found that there is a region in the binding site that binds $\pi$ electron systems. For example, the double bond of kainic acid (1) is important for high potency;[6] replacement with an aromatic substituent results in potent excitant substances,[7,8] and the compounds with the isopropenyl group replaced with a methyl ketone or an acid group are also active.[9]

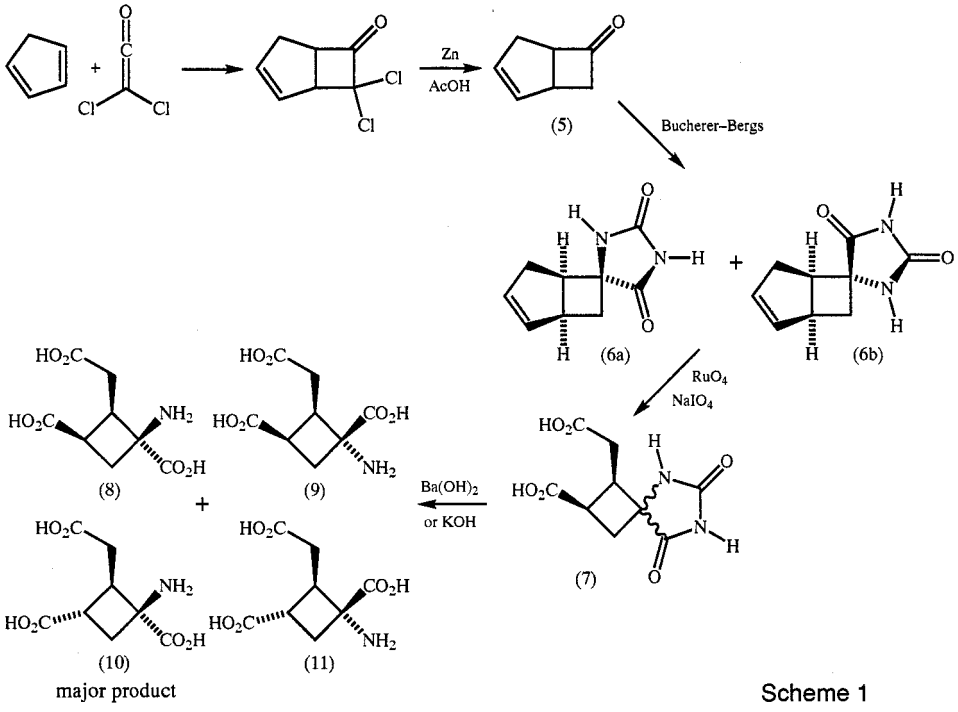
It seemed to us likely that a pocket with an affinity for $\pi$ electron systems may also exist in the NMDA receptor, and that such a pocket may be in a position somewhat similar to that in the kainic acid receptor subtype. The cyclobutane analogue (4) was chosen as a target compound after consideration of the relative position of the extra $\pi$ electron system of the acetic acid substituent by computer modelling. Without considering optical isomers, there are four cyclobutane stereoisomers possible, and these isomers hold the polar substituents in different but closely related spatial arrangements.

## Results and Discussion

The synthetic route to (4) (Scheme 1) involved dichloroketene addition to cyclopentadiene, and reduction to the intermediate bicyclo[3.2.0]hepten-6-one (5),[10] which is also available in a resolved form[11] should more detailed structure–activity investigations be warranted. Bucherer–Bergs reaction yielded the two isomeric hydantoins (6a) and (6b) in a 5:3 ratio in moderate yield. Recrystallization was monitored by $^1$H n.m.r. spectroscopy ($\delta$ 5·83 for major isomer, $\delta$ 5·72 for minor isomer), and gave an 8:1 mixture of major and minor isomers with reasonable recovery. Further crystallization produced the pure major isomer but in very low yield.

[4] Ishida, M., Ohfune, Y., Shimada, Y., Shimamoto, K., and Shinozaki, A., *Brain Res.*, 1991, **550**, 152.
[5] Allan, R. D., Hanrahan, J. R., Hambley, T. W., Johnston, G. A. R., Mewett, K. N., and Mitrovic, A. D., *J. Med. Chem.*, 1990, **33**, 2905.
[6] Watkins, J. C., in 'Kainic Acid as a Tool in Neurobiology' (Eds E. G. McGeer, J. W. Olney and M. D. McGeer) p. 37 (Raven Press: New York 1978).
[7] Hashimoto, K., Horikawa, M., and Shiramama, H., *Tetrahedron Lett.*, 1990, **31**, 7047.
[8] Hashimoto, K., and Shirahama, H., *Tetrahedron Lett.*, 1991, **32**, 2625.
[9] Anand, H., Roberts, P. J., Badman, G., Dixon, A. J., and Collins, J. F., *Biochem. Pharmacol.*, 1986, **35**, 409.
[10] Grieco, P. A., *J. Org. Chem.*, 1972, **37**, 2363.
[11] Corey, E. J., and Carpino, P., *Tetrahedron Lett.*, 1990, **31**, 7555.

For the oxidation step, the hydantoin was retained to act as a protecting group for the $\alpha$-amino acid group. Oxidation of the carbon–carbon double bond in the presence of the hydantoin NH giving the dicarboxylic acid (7) was achieved with a ruthenium tetraoxide catalysed system.[12] Early attempts with the catalyst in a water/acetone solvent mixture yielded dark ruthenium precipitates and starting material only. Inactivation of the ruthenium catalyst due to the formation of lower-valent ruthenium carboxylate complexes was assumed responsible. Addition of acetonitrile overcame this problem by redissolving the carboxylate complex and allowing the ruthenium to return to the catalytic cycle.[13] Greatly improved yields of the cleaved derivatives resulted when the ruthenium tetraoxide was generated in the carbon tetrachloride/acetonitrile/water solvent system.




Scheme 1

Hydrolysis of the hydantoin intermediate mixture caused considerable difficulty, and attempted derivatization of the expected amino acid products (8)–(11) for separation and purification as *N*-t-butoxycarbonyl, *N*-acetyl and/or methyl ester derivatives gave either more complex mixtures or poor conversions. The amino acid products were therefore characterized by t.l.c. ($R_F$ in butanol/acetic acid/water, 4:1:1), by $^1$H n.m.r. and $^{13}$C n.m.r. spectroscopy in an acidic medium, and by mass spectroscopy.

Initially it was found that refluxing the hydantoin mixture with potassium hydroxide for 2 days gave a mixture of isomeric amino acids in 20% yield, together with incompletely hydrolysed material. However, treatment of these

[12] Carroll, F. I., and Philip, A., *Org. Prep. Proced. Int.*, 1970, **2**, 223.
[13] Carlsen, P. H., Katsuki, I., Martin, V. S., and Sharpless, K. B., *J. Org. Chem.*, 1981, **46**, 3936.

residues again with potassium hydroxide or preferably with barium hydroxide[14] under reflux for several days resulted in the predominant formation of a single isomer of the amino acid. The same crystalline amino acid product was formed when the purified hydantoin intermediate (6) was oxidized and hydrolysed with potassium hydroxide as described above.

An attempt was made to isolate and characterize the other amino acids formed in the hydrolysis by using the Dowex 50 ($H^+$) cation-exchange chromatography technique which had successfully isolated (3).[5] Three fractions ($R_F$ 0·30, 0·38 and 0·30′ in order of elution) containing isomeric amino acids were separated for biological assay. Insufficient of the first fraction was isolated for complete characterization, and it may have been derived from a small amount of impurity with the double bond in the alternative position in the cyclopentene ring. The second fraction ($R_F$ 0·38) contained a mixture of two compounds, and was substantially isomerized by sodium hydroxide to another mixture with $R_F$ 0·30. The third fraction ($R_F$ 0·30′) was the major fraction, and was the only one to crystallize. It was identical to the crystalline amino acid from the barium hydroxide hydrolysis.

X-Ray analysis revealed the structure of the major crystalline product had a configuration with the 1,3-carboxylic acid groups on the ring *cis* to each other, and the carboxymethyl substituent *trans* to these acids, as shown in structure (10) and in Fig. 1. An explanation of the result is that the major hydantoin has the configuration shown in (6a); epimerization of the carboxylic acid substituent on position 3 of the cyclobutane ring occurs reasonably readily during hydrolysis of the hydantoin intermediate, and the resultant mixture contains relatively small amounts of the original isomers with the 2-acetic acid and 3-carboxy groups *cis* to each other. The second fraction ($R_F$ 0·38) is tentatively assigned as a mixture of (8) and (9) which mostly rearranges to (10) and (11) ($R_F$ 0·30) on base treatment.

**Table 1. Positional parameters for $C_8H_{11}NO_6.H_2O$ (10)**

| Atom | $10^4x$ | $10^4y$ | $10^4z$ | Atom | $10^4x$ | $10^4y$ | $10^4z$ |
|---|---|---|---|---|---|---|---|
| C(1) | 6842(3) | 3283(2) | 858(1) | C(6) | 8633(4) | 1238(2) | 901(1) |
| C(2) | 7291(3) | 2135(2) | 424(1) | C(7) | 7311(3) | 691(2) | 1613(1) |
| C(3) | 8399(3) | 2738(2) | −355(1) | O(3) | 8369(3) | −162(1) | 1969(1) |
| C(4) | 7193(3) | 3815(2) | −56(1) | O(4) | 5456(3) | 1005(1) | 1825(1) |
| N(1) | 8671(3) | 3629(2) | 1485(1) | C(8) | 7845(3) | 2369(2) | −1274(1) |
| C(5) | 4536(3) | 3454(2) | 1276(1) | O(5) | 8987(3) | 2619(1) | −1896(1) |
| O(1) | 4482(2) | 3709(1) | 2079(1) | O(6) | 5999(3) | 1779(2) | −1349(1) |
| O(2) | 2847(2) | 3345(1) | 784(1) | $O_W$ | 7377(3) | 5819(1) | 1698(1) |

The X-ray structure of the major crystalline product showed a zwitterionic molecule with a water molecule of hydration. The ORTEP plot[15] is illustrated in Fig. 1. Positional parameters, selected bond lengths and bond angles are presented in Tables 1–3. There were no intramolecular hydrogen bonds. Hydrogen atoms were reliably located and thus it could be established that the amine group

[14] Grunewald, G. L., Kuttab, S. H., Pleiss, M. A., and Mangold, J. B., *J. Med. Chem.*, 1980, **23**, 754.

[15] Johnston, C. K., ORTEP, A Thermal Ellipsoid Plotting Program, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1965.

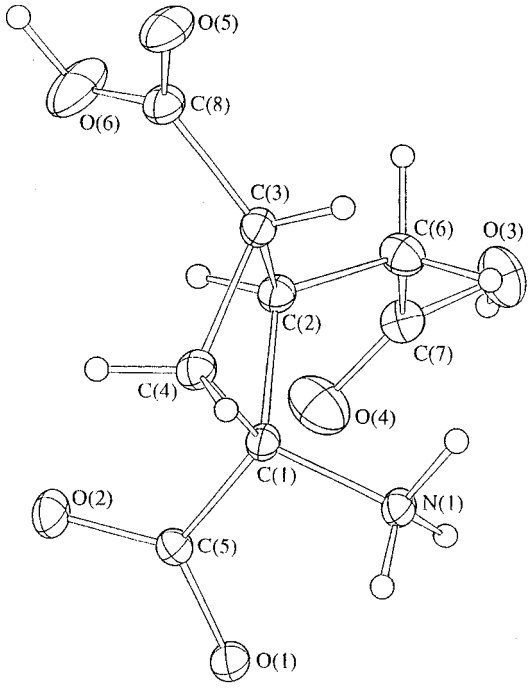



**Fig. 1.** ORTEP plot of X-ray structure of amino acid (10).

**Table 2. Bond lengths for $C_8H_{11}NO_6.H_2O$ (10)**

| Atoms | Distance (Å) | Atoms | Distance (Å) |
|---|---|---|---|
| C(2)–C(1) | 1·556(3) | C(4)–C(1) | 1·550(3) |
| N(1)–C(1) | 1·487(2) | C(5)–C(1) | 1·523(3) |
| C(3)–C(2) | 1·544(3) | C(6)–C(2) | 1·516(3) |
| C(4)–C(3) | 1·550(3) | C(8)–C(3) | 1·498(3) |
| O(1)–C(5) | 1·261(2) | O(2)–C(5) | 1·242(2) |
| C(7)–C(6) | 1·497(3) | O(3)–C(7) | 1·314(3) |
| O(4)–C(7) | 1·205(3) | O(5)–C(8) | 1·210(2) |
| O(6)–C(8) | 1·301(2) | | |

**Table 3. Bond angles for $C_8H_{11}NO_6.H_2O$ (10)**

| Atoms | Angle (degrees) | Atoms | Angle (degrees) |
|---|---|---|---|
| C(4)–C(1)–C(2) | 87·6(1) | N(1)–C(1)–C(2) | 113·3(2) |
| N(1)–C(1)–C(4) | 110·8(2) | C(5)–C(1)–C(2) | 117·2(2) |
| C(5)–C(1)–C(4) | 117·0(1) | C(5)–C(1)–N(1) | 109·5(1) |
| C(3)–C(2)–C(1) | 89·3(1) | C(6)–C(2)–C(1) | 121·2(2) |
| C(6)–C(2)–C(3) | 118·7(2) | C(4)–C(3)–C(2) | 88·0(1) |
| C(8)–C(3)–C(2) | 119·4(2) | C(8)–C(3)–C(4) | 115·4(2) |
| C(3)–C(4)–C(1) | 89·3(1) | O(1)–C(5)–C(1) | 118·4(2) |
| O(2)–C(5)–C(1) | 116·3(2) | O(2)–C(5)–O(1) | 125·3(2) |
| C(7)–C(6)–C(2) | 112·8(2) | O(3)–C(7)–C(6) | 113·2(2) |
| O(4)–C(7)–C(6) | 122·8(2) | O(4)–C(7)–O(3) | 124·0(2) |
| O(5)–C(8)–C(3) | 122·9(2) | O(6)–C(8)–C(3) | 114·3(2) |
| O(6)–C(8)–O(5) | 122·7(2) | | |

was protonated, the carboxylic acid adjacent to it existed as the anion and the remaining two carboxylic acid groups were un-ionized. Each of the acid protons of the structure was involved in a single strong hydrogen bond. The cyclobutane ring was significantly distorted from planarity as indicated by intra-ring torsion angles of 18°.

## Biological Assay

The three fractions of amino acid products from the ion-exchange column were tested for agonist or antagonist activity at excitatory receptors in rat brain by using a modified version of the rat cortical wedge preparation.[5,16] The compounds were screened at 500 $\mu$M and their effects against the depolarization induced by NMDA (10 $\mu$M), quisqualic acid (5 $\mu$M) or $\alpha$-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (5 $\mu$M), and kainic acid (2 $\mu$M) were observed. The hydantoin intermediates and the partially hydrolysed hydantoin intermediates were also tested. All the compounds screened showed no agonist activity (depolarization) while the first and second amino acids fractions displayed very weak and non-specific antagonist activity, for example reducing the depolarization due to NMDA and AMPA by about 20%. The most interesting pharmacological finding was that the second amino acid fraction [for which structures (8) and (9) are proposed] significantly decreased the rate of spontaneous firing of the tissue in the $Mg^{2+}$-deficient buffer. However, because of the difficulties in separating workable quantities of the pure isomers and the high concentrations needed for the effects the pharmacology of these compounds was not pursued further.

Despite the similarity in structure between the amino acid products and the potent NMDA agonist (3) none of the new amino acids showed even weak agonist activity at excitatory amino acid receptors. An explanation could be one of two possibilities: either the extra acetic acid functional group occupies a position of steric hindrance at the receptor binding site; or the cyclobutane ring may be prevented from adopting an appropriate conformation for receptor activation by the effect of the acetic acid substituent. The substantial effect of the acetic acid side chain on the conformation of these amino acids is illustrated by the isomerization of the intermediate amino acid (8) to form (10) with the 3-carboxylic acid and the 2-acetic groups in a *trans* relationship.

## Experimental

Infrared spectra were recorded on a Perkin–Elmer 177 spectrophotometer and refer to Nujol mulls of solids. $^1$H n.m.r. spectra were recorded on a Bruker A200F instrument at 360·13 MHz. $^{13}$C n.m.r. spectra were recorded on a Jeol FX-90Q instrument at 22·5 MHz or on a Bruker A200F instrument at 50 MHz. The solvents used were either $CDCl_3$ plus 2–3 drops $(CD_3)_2SO$ [with $SiMe_4$ (tetramethylsilane) as internal standard], or, with $D_2O$, 1 M DCl or 2 M NaOD [with either dioxan or dss (2,2-dimethyl-2-silapentane-5-sulfonate) as an internal reference standard]. Low-resolution mass spectra data refer to chemical ionization with methane as the reagent gas on a TSQ46 Finnigan/MAT mass spectrometer. Microanalyses were determined by the Australian Microanalytical Service, Melbourne. $R_F$ values refer to thin-layer chromatography (t.l.c.) run on aluminium-backed Merck Kieselgel 60 $G_{254}$ plates and determined by using the solvent system as indicated. The plates were visualized with either iodine, permanganate or Ninhydrin spray. Ion-exchange chromatography was performed with Dowex 50W resin ($H^+$ form) by using either water or 1 M pyridine as the eluent, as indicated.

[16] Harrison, N. L., and Simmonds, M. A., *Br. J. Pharmacol.*, 1985, **84**, 381.

*Bicyclo[3.2.0]hept-2-en-6-one (5)*

The dichloro derivative, which proved unstable to storage, was prepared by a procedure similar to that of Grieco,[10] cyclopentadiene (103 g, 1·56 mol) and freshly distilled dichloroacetyl chloride (46 g, 0·31 mol) with triethylamine (47 g, 0·47 mol) yielding 7,7-dichlorobicyclo[3.2.0]hept-2-en-6-one (44 g, 80%) as a clear liquid, b.p. 58–61°/0·25 mmHg (lit. 49–50°/0·3 mmHg).

This dichloro derivative (44 g, 0·25 mol) was reduced with zinc dust (40 g, 0·61 mol) according to the published method[10] to yield bicyclo[3.2.0]hept-2-en-6-one (5) (13·2 g, 50%) as a clear liquid, b.p. 64–68°/25 mmHg (lit. 60°/15 mmHg).

*Bicyclo[3.2.0]hept-2-ene-6-spiro-5′-hydantoin (6)*

To a solution of enone (5) (5 g, 46 mmol) in 1:1 methanol/water (100 ml) were added potassium cyanide (7·65 g, 118 mmol) and ammonium carbonate (23 g, 290 mmol), and the reaction mixture was heated at 50–55° for 5 days. The cooled reaction mixture was then evaporated under reduced pressure to remove the methanol. The solution was subjected to continuous extraction with ether (250 ml) for 2 days. The ether layer was then washed with water (50 ml) and brine, and dried ($Na_2SO_4$). Evaporation of the ether layer under reduced pressure afforded the crude hydantoin product as a white powder which was recrystallized from ethyl acetate to yield *bicyclo[3.2.0]hept-2-ene-6-spiro-5′-hydantoin* as a mixture of (6a) and (6b) (4·8 g, 58%) with $R_F$ 0·25 and 0·16 (ethyl acetate/toluene, 1:1). Fractional crystallization from ethyl acetate yielded a small amount of the major isomer as long, white rods, $R_F$ 0·25, m.p. 223–225° (Found: C, 60·8; H, 5·4; N, 15·6. $C_9H_{10}N_2O_2$ requires C, 60·7; H, 5·6; N, 15·7%). $\nu_{max}$ (Nujol) 3400–3050w, 1770s, 1730s, 1660sh $cm^{-1}$. $^1H$ n.m.r. $\delta$ [$CDCl_3$, $(CD_3)_2SO$] 1·91, d, $J$ 12·5 Hz, 1H, H7; 2·51, m, 2H, H4; 3·00–3·08, m, 1H, H7; 3·17, br m, 1H, H1 or H5; 3·45, br m, 1H, H5 or H1; 5·82 and 5·84, overlapping s, H2 and H3; 6·28, br s, NH; 9·60, br s, NH. For the minor isomer, $\delta$ 5·72, m, H2 and H3, was distinctive. Mass spectrum $m/z$ 179 (MH, 100%), 164 (5), 136 (12), 113 (10).

*3-Carboxy-2-(carboxymethyl)cyclobutane-1-spiro-5′-hydantoin (7)*

The mixture of isomeric unsaturated hydantoins (6) (0·5 g, 2·8 mmol) was dissolved in acetonitrile (10 ml), and A.R. carbon tetrachloride (10 ml) added to the stirred solution. A solution containing sodium metaperiodate (2·46 g, 11·5 mmol) in water (15 ml) was added, and to this vigorously stirring reaction mixture was added a catalytic amount of ruthenium trichloride hydrate (14 mg, 0·06 mmol). On this last addition, the colour immediately became a deep orange; after further stirring for 2–3 min, the colour faded to a bright yellow and a white precipitate began to form. After stirring rapidly at room temperature for 4 h, t.l.c. (methanol/ethyl acetate, 1:5) indicated no starting material present. Isopropyl alcohol (30 ml) was then added to terminate the reaction, and the solid was filtered off and washed with isopropyl alcohol. The filtrate was evaporated under reduced pressure, water (20 ml) added and the product extracted into ethyl acetate (5×20 ml). The combined organic layer was washed with brine, and dried ($Na_2SO_4$). The organic layer was evaporated under reduced pressure to afford the *hydantoin* (7) as a white solid (0·63 g, 92%), m.p. 216–219° (Found: C, 44·7; H, 4·3; N, 11·4. $C_9H_{10}N_2O_6$ requires C, 44·6; H, 4·1; N, 11·6%). $\nu_{max}$ (Nujol) 3500–3000w, 1765s, 1730s, 1700sh $cm^{-1}$. $^1H$ n.m.r. $\delta$ [$CDCl_3$, $(CD_3)_2SO$] 2·47, dd, $J$ 4·8, 12·8 Hz, H4; 2·50, dd, $J$ 5·8, 17·5 Hz, 1H, $CH_2COOH$; 2·59, dd, $J$ 9, 17·5 Hz, 1H, $CH_2COOH$; 2·76, dd, $J$ 9, 12·6 Hz, H4; 3·19, ddd, $J$ 9, 9, 4·8 Hz, H3; 3·52, ddd, $J$ 5·8, 9, 9 Hz, H2; 7·18, s, NH; 9·89, s, NH. The minor isomer had peaks at $\delta$ 3·00, dd, $J$ 5, 12 Hz; 3·33, m; 7·82, s, NH; 9·74, s, NH. $^{13}C$ n.m.r. $\delta$ ($D_2O$, dioxan) 31·6, 31·9, 32·0, 32·2, 2×($CH_2$, $CH_2CO_2H$); 35·1, 36·2, 41·5, 42·9, 2×(C2, C3); 63·7, 63·9, 2×C1; 159·7, 176·3, 176·8, 177·0, 177·6, 180·8, C=O. Mass spectrum $m/z$ 243 (MH, 100%), 225 (43), 113 (12).

*1-Amino-2-(carboxymethyl)cyclobutane-1,3-dicarboxylic Acids*

*Part* A. The isomeric mixture of 3-carboxy-2-(carboxymethyl)cyclobutane-1-spiro-5′-hydantoin (3·2 g, 13·22 mmol) was dissolved in 2 M potassium hydroxide (53 ml), and

the solution refluxed for 2 days. The white inorganic precipitate was removed by filtration and the filtrate concentrated under vacuum. The crude material was purified by chromatography through Dowex 50W ($H^+$) ion-exchange resin (150 ml) with water as the initial eluent to give fractions that were used in part B. After the fractions became neutral, the eluent was gradually changed to 1 M pyridine. Fractions of 50 ml were collected and examined by t.l.c. (Ninhydrin spray) for combination. Evaporation under reduced pressure yielded a mixture of amino acids (0·63 g, 22%), $R_F$ 0·38, 0·30 (butanol/acetic acid/water, 4:1:1). Recrystallization from water afforded a small sample of one pure isomer of 1-amino-2-(carboxymethyl)cyclobutane-1,3-dicarboxylic acid as white rods ($R_F$ 0·30). This product was identical by t.l.c., and $^1H$ n.m.r., to the pure amino acid isolated in the procedure described in part B below.

*Part* B. The aqueous fractions from the Dowex 50W column were combined and concentrated, and the mixture was hydrolysed with barium hydroxide (160 ml, 0·3 M). After refluxing for 3 days, the reaction mixture was cooled, acidified with 6 M HCl, and filtered through Celite. The filtrate was concentrated and applied onto a Dowex 50W ($H^+$) ion-exchange column (150 ml), and eluted as described above. The appropriate pyridine fractions were combined and evaporated to afford a pure amino acid (0·92 g, 32%). The product was recrystallized a number of times from water for characterization and submission for X-ray analysis, giving *(1β,2β,3α)-1-amino-2-(carboxymethyl)cyclobutane-1,3-dicarboxylic acid hydrate* (10) as white rods, $R_F$ 0·30 (butanol/acetic acid/water, 4:1:1; Ninhydrin spray), m.p. 153–154° (Found: C, 41·1; H, 5·6; N, 6·2. $C_8H_{11}NO_6.H_2O$ requires C, 40·8; H, 5·5; N, 5·9%). $\nu_{max}$ (Nujol) 3550–3350w, 3100–2500w, 1705sh, 1675s, 1590, 1400s $cm^{-1}$. $^1H$ n.m.r. $\delta$ ($D_2O$, 2 M NaOD, dss) 1·69–1·76, m (7 lines), 1H, H4; 2·31, 2·34, 2×apparent s, C**H**$_2$COOH; 2·71–2·77, m, 2H, H4′ and H2; 3·29–3·36, m (4 lines), 1H, H3. $^1H$ n.m.r. $\delta$ ($D_2O$, 1 M DCl, dss) (couplings confirmed by decoupling experiments) 2·57, dd, $J_{H4,H4'}$ 13·6, $J_{H4,H3}$ 9·6 Hz, H4; 2·72, dd, $J_{H\alpha,H\alpha'}$ 17·6, $J_{H\alpha,H2}$ 9·6 Hz, H$\alpha$; 2·82, dd, $J_{H\alpha',H\alpha}$ 17·6, $J_{H\alpha',H2}$ 6·5 Hz, H$\alpha'$; 3·00, dd, $J_{H4',H4}$ 13·6, $J_{H4',H3}$ 9·6 Hz, H4′; 3·31, dd, $J_{H3,H4}$ 9·6, $J_{H3,H2}$ 9·6 Hz, H3; 3·71, ddd, $J_{H2,H\alpha'}$ 6·5, $J_{H2,H\alpha'}$ 9·6, $J_{H2,H3}$ 9·6 Hz, H2. $^{13}C$ n.m.r. $\delta$ ($D_2O$, 1 M DCl, dioxan, 50 MHz) 30·8, 32·3, $CH_2$, C**H**$_2$COOH; 37·0, C**H**CH$_2$COOH; 39·8, C**H**COOH; 58·1, C1; 171·7, 174·4, 176·1, 3×C=O. Mass spectrum *m/z* 218 (MH, 3%), 200 (100), 182 (18), 154 (23). When the 8:1 isomeric mixture of bicyclo[3.2.0]hept-2-ene-6-spiro-5′-hydantoins was isolated and treated separately by ruthenium tetraoxide oxidation and barium hydroxide hydrolysis (as described above), one isomer was isolated, identical to the product described above.

*Part* C. The crude amino acid product (300 mg) from part A which contained a mixture of isomers was reapplied onto a Dowex 50W ($H^+$) ion-exchange column (100 ml), and the column eluted with water. After approximately 1200 ml, Ninhydrin-positive fractions appeared and 20-ml fractions were subsequently collected, monitored by t.l.c. (butanol/acetic acid/water, 4:1:1; Ninhydrin spray) and combined appropriately. Although considerable overlap occurred, only pure fractions were combined to give a series of three Ninhydrin-positive fractions, the order of elution from the column being $R_F$ 0·30, 0·38 and 0·30′ respectively. The major product was present in the third fraction (designated here as $R_F$ 0·30′ to differentiate it from the other product of $R_F$ 0·30). Only a very small quantity (*c.* 5 mg) of the first fraction was isolated. The $^1H$ n.m.r. spectrum of each of the three fractions was different, and the third fraction ($R_F$ 0·30′) corresponded to the pure amino acid isolated in part B above. These three fractions were characterized by n.m.r. and mass spectroscopy (see below).

$R_F$ 0·30. $^1H$ n.m.r. $\delta$ ($D_2O$, 1 M DCl, dss) 2·51, dm, H4; 2·71, 2·74, 2×s, C**H**$_2$COOH; 2·86–3·00, m, 1H, H2, H3 or H4′; 3·43–3·54, m, 1H, H2, H3 or H4′; 3·73–3·78, m, 1H, H2, H3 or H4′. $^{13}C$ n.m.r. $\delta$ ($D_2O$, 1 M DCl, dioxan, 50 MHz) 29·8, 30·3, $CH_2$, **C**H$_2$COOH; 36·6, **C**HCH$_2$COOH; 37·8, **C**HCOOH; 58·8, C1; 172·1, 174·4, 177·5, 3×C=O (a minor contaminant was also present). Mass spectrum *m/z* 218 (MH, 5%), 200 (55), 113 (100).

$R_F$ 0·38. $^1H$ n.m.r. $\delta$ ($D_2O$, 2 M NaOD, dss): isomerization occurred at room temperature overnight giving a spectrum identical to that assigned to (10) in part B together with a second significant product: 1·83–1·93, m, H4; 2·21–2·36, m, 2×H$\alpha$; 2·53–2·68, m, H4′, H2, H3. $^1H$ n.m.r. $\delta$ ($D_2O$, 1 M DCl, dss) (2:3 mixture of two isomers designated A and B) 2·59–2·71, m, H4(A+B); 2·80–2·95, m, H4′(A+B), 2×H$\alpha$(B); 3·05–3·10, m, 2×H$\alpha$(A); 3·24, apparent q, *J* 9·4 Hz, H2 or H3(A); 3·41, apparent q, *J* 8·5 Hz, H3 or H2(A); 3·55–3·66, m, H2(B), H3(B). $^{13}C$ n.m.r. $\delta$ ($D_2O$, 1 M DCl, dioxan, 50 MHz) 29·4, 30·8, 31·8, 34·0, 2×$CH_2$, 2×**C**H$_2$COOH; 34·6, 37·1, 2×**C**HCH$_2$COOH; 39·4, 41·9, 2×**C**HCOOH;

59·0, C1; 171·3, 171·5, 171·8, 175·3, 175·4, 175·6, 6×C=O. Mass spectrum *m/z* 218 (MH, 100%), 200 (78), 182 (12), 172 (30), 154 (15).

$R_F$ 0·30′. Identical to the product isolated above in part B.

*Crystallography*

For diffractometry a crystal of dimensions 0·20 by 0·30 by 0·32 mm was mounted on a glass fibre with cyanoacrylate resin. Lattice parameters at 21°C were determined by a least-squares fit to the setting parameters of 25 independent reflections, measured and refined on an Enraf–Nonius CAD4F four-circle diffractometer employing graphite-monochromatized Mo Kα radiation.

*Crystal data.* Formula $C_8H_{13}NO_7$; $M$ 235·20, monoclinic, space group $P2_1/c$, $a$ 5·8869(14), $b$ 12·030(2), $c$ 15·230(3) Å, $\beta$ 90·96(2)°; $V$ 1078·4(4) Å$^3$, $Z$ 4, $D_c$ 1·449 g cm$^{-3}$, $\mu$(Mo Kα) 0·85 cm$^{-1}$, $\lambda$(Mo Kα) 0·7107 Å, $F$(000) 496 e.

Intensity data were collected in the range $1 < \theta < 25°$ by using an $\omega$–$2\theta$ scan. Data reduction and application of Lorentz and polarization corrections were carried out with the Enraf–Nonius Structure Determination Package.[17] Of the 2044 independent non-zero reflections collected, 1345 with $I > 2{\cdot}5\sigma(I)$ were considered observed and used in the calculations. The structure was solved by direct methods with SHELXS-86,[18] and the solution was extended by difference-Fourier methods. Hydrogen atoms were refined with isotropic thermal parameters and all other atoms were refined anisotropically. Upon full-matrix least-squares refinement of an overall scale factor, positional and thermal parameters converged (all shifts $<0{\cdot}002\sigma$) with residuals* $R$ 0·033 and $R_w$ 0·038, and $w = 1{\cdot}57/(\sigma^2(F_o)+0{\cdot}00025F_o^2)$. Maximum excursions in a final difference map were +0·23 and −0·17 e Å$^{-3}$. Scattering factors and anomalous dispersion terms used were those supplied in SHELX-76.[19] All calculations were carried out with SHELX-76 and plots were drawn with ORTEP.[15]

The atom numbering scheme is given in Fig. 1. Final atomic coordinates, bond lengths and bond angles are listed in Tables 1–3. Listings of observed and calculated structure factors, torsion angles, non-hydrogen atom thermal parameters, hydrogen atom coordinates and thermal parameters, close non-bonded contacts, and details of least-squares planes calculations are deposited as an Accessory Publication.†

*Rat Cortical Wedge Preparation*

Slices of rat neocortex and corpus callosum were obtained from Sprague–Dawley rats weighing 200–300 g, as previously described.[5,16] Cerebral wedges were prepared and placed between layers of absorbent fibre supported on an inclined block at room temperature, superfused with a magnesium-free Krebs solution oxygenated with 95% oxygen and 5% carbon dioxide, at a rate of 1 ml/min. Direct-current potentials between the cingulate cortex and corpus callosum were monitored by Ag/AgCl electrodes via agar/saline bridges and a high-impedence direct-current amplifier, and were displayed on a chart recorder. All compounds tested were made up in magnesium-free Krebs buffer, and were applied to the cortex for periods of 1 min when testing for agonist activity or 5 min when testing for antagonist activity. With this arrangement, recording cortex against corpus callosum, activity was measured either as a depolarizing response (an upward deflection from the baseline where amplitude was measured in mV) or as an effect on the repetitive spontaneous discharge.

* $R = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$, $R_w = (\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2)^{1/2}$.

† Copies are available on application to the Australian Journal of Chemistry, P.O. Box 89, East Melbourne, Vic. 3002.

[17] Enraf–Nonius Structure Determination Package (SDP), Enraf Nonius, Delft, Holland, 1985.
[18] Sheldrick, G. M., SHELXS-86, in 'Crystallographic Computing 3' (Eds G. M. Sheldrick, C. Kruger and R. Goddard) p. 175 (Oxford University Press: Oxford, England, 1985).
[19] Sheldrick, G. M., SHELX-76, in 'A Program for X-Ray Crystal Structure Determination' (University of Cambridge: Cambridge, England, 1976).

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