Studies on Pyrrolidinones. Synthesis of some *N*-Fatty Acylpyroglutamic Acids

Benoît Rigo*

Laboratoire Chimie Organique et Environnement, Ecole des Hautes Etudes Industrielles, 13 rue de Toul, 59046 Lille Cedex, France

Antonios Kolokouris and Nicolas Kolokouris*

Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimioupoli-Zografou GR-15771, Athens, Greece

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Starting from readily available pyroglutamic acid **1**, some *N*-fatty acylpyroglutamic acids were synthesized and characterized by their spectral data. A preliminary pharmacological study showed that *N*-stearoylpyroglutamic acid **4c** displays a CNS stimulating effect on mice.

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Pyroglutamic acid **1** has been called "the forgotten amino acid" [1], but with some of its derivatives, it occurs in a large number of animal tissues; they are found as the amino-terminal residue in many biologically active neuropeptides [2] and are especially concentrated in the skin [3].

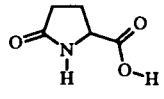

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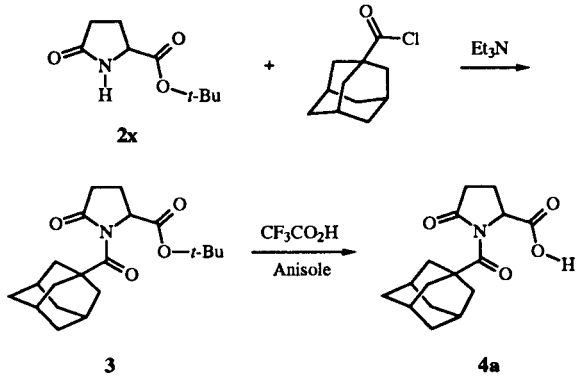
Figure 1

In this context, pyroglutamic acid is present in substantial amount in the brain [4], where it is several thousand times less neurotoxic than kainic acid [5]; as for the memory increase, D-pyroglutamic acid is more efficient than the L-acid [6]. Pyroglutamic acid is involved with glutathione metabolism [7], and 5-oxoprolinase converts pyroglutamic acid into glutamate [8], a major excitatory amino acid neurotransmitter in the central nervous system [9]. Because of its hydrophilic nature, pyroglutamic acid does not cross the blood-brain barrier and does not reach the brain; on the other hand, it is known that fatty acids cross this barrier [10-14] and that the linking of a fatty acid to an aminoacid helps it to reach the brain [15-18]. These observations, the great number of biological properties found for *N*-acylpyroglutamic derivatives [19-20], *N*-acylpyrrolidinone [21] and *N*-fatty acylglutamic acids [22], and our interest in pyroglutamic acid [23] and adamantane [24] chemistry, prompted us to realize the synthesis of pyroglutamic derivatives *N*-substituted by a fatty acid or an adamantane carbonyl group, in order to test the ability of these compounds to be used as a prodrug of pyroglutamic acid, in the same way as *N*-acyl-2-pyrrolidinone can be used as a prodrug of Gaba because its biological degradation first yields *N*-acylaminobutyric acid, then Gaba [25-26].

While in some cases [21] the acylation of pyroglutamic esters by acyl chlorides in the presence of triethylamine gives poor yields in *N*-acyllactame [27], by using this method it was possible to obtain *t*-butyl *N*-adamantanecarbonylpyroglutamate **3** in 42% yield; treatment of this ester with trifluoracetic acid quantitatively gives the *N*-acyl acid **4a** (Scheme 1).

Scheme 1

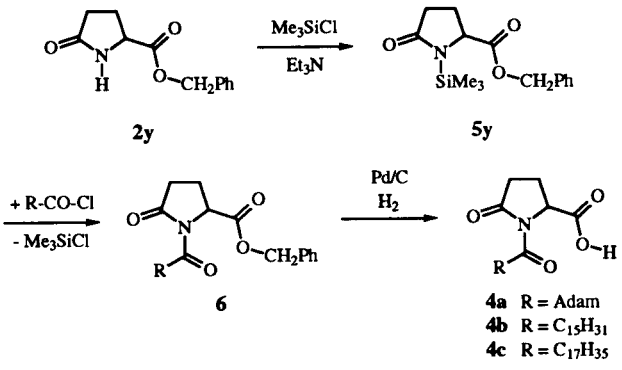


A more general method to obtain these *N*-acyl derivatives was the reaction of benzyl *N*-trimethylsilylpyroglutamate **2y** with acyl chlorides; hydrogenolysis of esters **6** quantitatively yields acids **4** (Scheme 2).

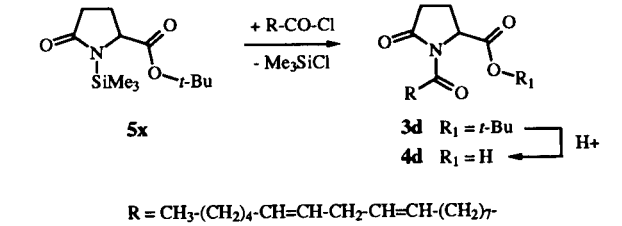
It was not possible to obtain *N*-linoleylpyroglutamic acid by this hydrogenolytic method. This difficulty was overcome by using the reaction of *N*-silyl esters **5x** with linoleyl chloride (Scheme 3).

Another way to avoid the hydrogenolysis step was to react *N,O*-bistrimethylsilylpyroglutamic acid **7** with acyl chlorides. This reaction gives a very good yield in silyl esters **8**, and the addition of methanol to the reaction medium hydrolyzed rapidly these esters into acids **4** (Scheme 4).

Scheme 2

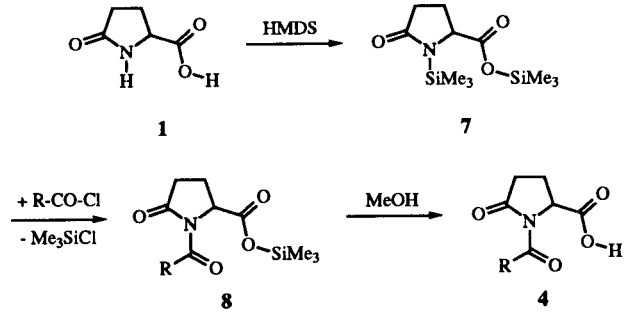


Scheme 3



R = CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-$(CH_2)_7$-

Scheme 4



A preliminary pharmacological study of the action of acid **4c** on mice has been realized: at 100 mg/Kg (*via* intramuscular injection) a strong increase of motility has been observed, and at 200 mg/Kg it causes fear and a strong stereotype. This central nervous system stimulating effect could be induced by the glutamic acid coming from hydrolysis of the prodrug **4c** [28].

The biological properties of the other esters and acids **3**, **4** and **6** will be reported later.

EXPERIMENTAL

Melting points were determined using a Büchi capillary apparatus and are uncorrected; the ir spectra were recorded on a Perkin-Elmer 883 spectrometer and the nmr spectra on a Bruker AC 200 at 200 MHz or on a Hitachi Perkin-Elmer R-600 at 60 MHz, using tetramethylsilane as an internal reference. Elemental analyses were performed by the "Service Central de Microanalyses" of CNRS in Vernaison, France.

t-Butyl *N*-Adamantanecarbonylpyroglutamate (**3**).

A solution of 1-adamantanecarbonyl chloride (1.1 g, 0.0054 mole) in dry tetrahydrofuran (10 ml), was added to an ice cooled mixture of *t*-butyl pyroglutamate **2x** [19] (1.5 g, 0.008 mole) and triethylamine (0.8 g, 0.008 mole) in dry tetrahydrofuran (10 ml). The solution was refluxed for 2 hours, the precipitate of triethylamine hydrochloride was filtered and the solvent evaporated. Ether (40 ml) was added, the solution was washed with water and brine. After drying, the solution was filtered on a basic aluminum oxide bed. The residue obtained after evaporation crystallized in a pentane/ether mixture, yield 0.8 g, 43%, mp 143-145° (ether-pentane); ir (nujol): ν cm^{-1} 1748, 1730, 1660, 1273, 1240; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 1.42 (s, 9H), 1.60-1.71 (m, 6H), 1.95-2.14 (m, 9H), 1.90-2.80 (m, 4H), 4.55 (dd, J = 2.7, 9.6 Hz, 1H).

Anal. Calcd. for $C_{20}H_{29}NO_4$: C, 69.14; H, 8.41; N, 4.03. Found: C, 69.03; H, 8.41; N, 3.89.

Benzyl Pyroglutamate (**2y**).

A stirred mixture of pyroglutamic acid (30 g, 0.233 mole), benzyl alcohol (36.7 g, 0.349 mole) and *p*-toluenesulfonic acid (1.5 g) was refluxed for 40 hours in a Dean-Starck apparatus. Methylene dichloride (300 ml) was added and the solution was washed with a sodium hydrogen carbonate solution then with brine. After drying and evaporation, the product was distilled, yield 36.3 g, 71%; this product displayed the same physical properties as the one given in ref [19], mp 50-52° (ether), bp 185° (0.1 mm Hg); ir (nujol): ν cm^{-1} 3400, 3170, 3080, 1735, 1670, 1600, 1500, 1185; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 2.1-2.7 (m, 4H), 4-4.5 (m, 1H), 5.14 (s, 2H), 6.79 (s, 5H), 7.31 (s, 1H), deuterium oxide exchangeable. It was important to dry perfectly the organic solutions of the benzyl ester before distilling, otherwise there was formation of hydrates which partly decomposed during the distillation.

Benzyl *N*-trimethylsilylpyroglutamate (**5y**).

A solution of benzyl pyroglutamate (**2y**) (19.5 g, 0.089 mole) in toluene (60 ml) and triethylamine (10.8 g, 0.106 mole) was refluxed, then trimethylchlorosilane (11.6 g, 0.107 mole) was slowly added. The refluxing was continued for 4 hours, and after cooling at room temperature, the precipitate of triethylamine hydrochloride was filtered and washed with toluene. The solvent was evaporated and the residue distilled, yield 21.9 g, 85%, bp 150° (0.05 mm Hg); ^1H nmr (60 MHz) (deuteriochloroform): δ ppm 0.23 (s, 9H), 2.1-2.6 (m, 4H), 4.1-4.4 (m, 1H), 5.22 (s, 2H), 7.36 (s, 5H).

Benzyl *N*-Adamantanecarbonylpyroglutamate (**6a**).

A solution of benzyl *N*-trimethylsilylpyroglutamate (**5y**) (8.1 g, 0.029 mole) in tetrahydrofuran (20 ml) was refluxed, then 1-adamantanecarbonyl chloride (from 1-adamantanecarboxylic acid, 5 g, 0.028 mole, and thionyl chloride, 3.4 ml) in dry tetrahydrofuran (15 ml) was slowly added. The refluxing was continued for 7 hours, the solvent was evaporated and the residue was dissolved in ether then filtered on a basic aluminum oxide bed. The compound obtained after evaporation was crystallized in a 1/4 ether/pentane mixture, yield 10 g, 90%, mp 141-143° (benzene-pentane); ir (nujol): ν cm^{-1} 1750, 1742, 1660, 1278, 1228; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm

1.63 (br s, 6H), 1.78-2.01 (m, 9H), 2.04-2.71 (m, 4H), 4.69 (dd, J = 9.4, 2.6 Hz, 1H), 4.98 (d, J = 12.2 Hz, 1H), 5.15 (d, J = 12.2 Hz, 1H), 7.24 (s, 5H).

Anal. Calcd. for $C_{23}H_{27}NO_4$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.53; H, 7.15; N, 3.48.

Benzyl *N*-Palmitoylpyroglutamate (**6b**).

A solution of palmitoyl chloride (4.7 g, 0.017 mole) in dry tetrahydrofuran (10 ml) was slowly added to a solution of benzyl *N*-trimethylsilylpyroglutamate (**5y**) (5 g, 0.017 mole) in dry tetrahydrofuran (20 ml). The solution was refluxed for 5 hours, the solvent was evaporated, the residue was dissolved in ether, then filtered on a basic aluminum oxide bed. The compound obtained after evaporation of the solvents crystallized upon cooling, yield 7.2 g, 93%, mp 28-30° (pentane); ir (nujol): ν cm^{-1} 1740, 1736, 1685, 1280, 1223; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 0.92 (t, J = 6.1 Hz, 3H), 1.28 (br s, 24H), 1.61 (t, J = 7 Hz, 2H), 1.96-3.07 (m, 6H), 4.81 (dd, J = 9.3, 2.4 Hz, 1H), 5.14 (d, J = 12.4 Hz, 1H), 5.21 (d, J = 12.4 Hz, 1H), 7.36 (s, 5H).

Anal. Calcd. for $C_{28}H_{43}NO_4$: C, 73.49; H, 9.47; N, 3.06. Found: C, 73.29; H, 9.42; N, 2.98.

Benzyl *N*-Stearoylpyroglutamate (**6c**).

This compound, synthesized in the same way as for ester **6b**, crystallized when freezing, yield 90%, mp 37-38° (pentane); ir (nujol): ν cm^{-1} 1750, 1716, 1681, 1270, 1223; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 0.87 (t, J = 6.1 Hz, 3H), 1.25 (br s, 28H), 1.60 (t, J = 7 Hz, 2H), 1.94-2.89 (m, 6H), 4.80 (dd, J = 9.3, 2.4 Hz, 1H), 5.14 (d, J = 12.2 Hz, 1H), 5.22 (d, J = 12.2 Hz, 1H), 7.33 (s, 5H).

Anal. Calcd. for $C_{30}H_{47}NO_4$: C, 74.22; H, 9.75; N, 2.88. Found: C, 74.02; H, 9.97; N, 2.71.

N-Adamantanecarbonylpyroglutamic Acid (**4a**).

From Silyl Ester **7**.

A solution of trimethylsilyl *N*-trimethylsilylpyroglutamate (**7**) [22] (1.6 g, 0.0058 mole) in dry tetrahydrofuran (10 ml) was refluxed, then 1-adamantanecarbonyl chloride (from adamantanecarboxylic acid, 1 g, 0.0055 mole) in dry tetrahydrofuran (10 ml) was slowly added (2 hours). The refluxing was continued for 7 hours. After cooling, methanol (1 ml) and water (1 ml) were added, then the solvents were evaporated under vacuum. The residue was dissolved in dichloromethane, washed with water then evaporated. The residue crystallized at -12° in a 1/4 ether/pentane mixture, yield 0.95 g, 59%, mp 163-164° (acetone-pentane); ir (nujol): ν cm^{-1} 1745, 1703, 1678, 1285, 1222; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 1.75 (s, 6H), 2.04 (s, 3H), 2.12 (s, 6H), 2.05-2.90 (m, 4H), 4.77 (dd, J = 9.6, 2.7 Hz, 1H), 10.45 (br s, 1H).

Anal. Calcd. for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 66.03; H, 7.35; N, 4.57.

From Ester **3**.

t-Butyl ester **3** (0.4 g, 0.0012 mole) was added to a mixture of trifluoroacetic acid (5 ml) and anisole (1 ml). After stirring for 1 hour, the mixture was evaporated and the residue was recrystallized in an acetone/pentane mixture, yield 0.32 g, 97%.

From Ester **6a**.

A mixture of benzyl ester **6a** (2 g, 0.0053 mole) and 10% Pd-C (0.5 g) in ethanol (10 ml) was stirred under hydrogen (45 psi) for 3 hours. The residue obtained after filtration and evaporation was recrystallized in a pentane-acetone mixture, giving a near quantitative yield of acid **4a**.

N-Palmitoylpyroglutamic Acid (**4b**).

This compound was obtained from ester **6b** in the same way as for the acid **4a** (nearly quantitative yield) mp 76-78° (tetrahydrofuran/pentane); ir (nujol): ν cm^{-1} 1750, 1716, 1681, 1270, 1223; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 0.90 (t, J = 6.1 Hz, 3H), 1.29 (br s, 24H), 1.62 (t, J = 7 Hz, 2H), 2.09-3.07 (m, 6H), 4.78 (dd, J = 9.3, 2.4 Hz, 1H), 7.20 (br s, 1H).

Anal. Calcd. for $C_{21}H_{37}NO_4$: C, 68.63; H, 10.15; N, 3.81. Found: C, 68.53; H, 10.46; N, 3.69.

N-Stearoylpyroglutamic Acid (**4c**).

This compound was obtained from ester **6c** in the same way as for the acid **4a** (nearly quantitative yield), mp 77-79° (tetrahydrofuran/pentane); ir (nujol): ν cm^{-1} 1750, 1718, 1685, 1270, 1220; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 0.88 (t, J = 6.1 Hz, 3H), 1.25 (br s, 28H), 1.60 (t, J = 7 Hz, 2H), 2.05-3.01 (m, 6H), 4.74 (dd, J = 9.3, 2.4 Hz, 1H), 7.0 (br s, 1H).

Anal. Calcd. for $C_{23}H_{41}NO_4$: C, 68.83; H, 10.45; N, 3.54. Found: C, 68.63; H, 10.78; N, 3.61.

N-Linoleylpyroglutamic Acid (**4d**).

A solution of linoleyl chloride (3.1 g, 0.0103 mole) in dry tetrahydrofuran (10 ml) was slowly added to a solution of *t*-butyl *N*-trimethylsilylpyroglutamate (3.2 g, 0.0124 mole) [21] in dry tetrahydrofuran (10 ml). The solution was refluxed for 5 hours then evaporated. The residue was filtered on a neutral aluminum oxide bed (2/1 ether/pentane), giving 5 g, 90% yield of *t*-butyl *N*-linoleylpyroglutamate (**3d**). A solution of this ester (0.5 g, 0.0011 mole) in anisole (0.5 g) and trifluoroacetic acid (5 ml) was stirred for 1 hour then evaporated. The residue was dissolved in a mixture of ether and pentane then washed with a sodium hydrogen carbonate solution. Dilute hydrochloric acid was added to the aqueous solution, giving an oil. This oil was extracted with ether, washed with water, dried then evaporated under vacuum (40°, 0.001 mm Hg). The pure (tlc, 1/1 methanol/dichloromethane), air sensitive acid **4e** was obtained in a near quantitative yield; ir (nujol): ν cm^{-1} 1755, 1708, 1655, 1612, 1222; ^1H nmr (200 MHz) (deuteriochloroform): δ ppm 0.84 (t, J = 6 Hz, 3H), 1.26 (br s, 16H), 1.58 (br s, 2H), 1.90-3.0 (m, 10H), 4.72 (dd, J = 9.3, 2.4, 1H), 5.29 (m, 4H), 11.26 (br s, 1H).

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