Tetrahedron Letters Vol. 21, pp 2355 - 2358
0040-4039/80/0608-2355$02.00/0

SYNTHESIS OF NOVEL NEUROEXCITATORY AMINO ACIDS DERIVED FROM KAINIC ACID

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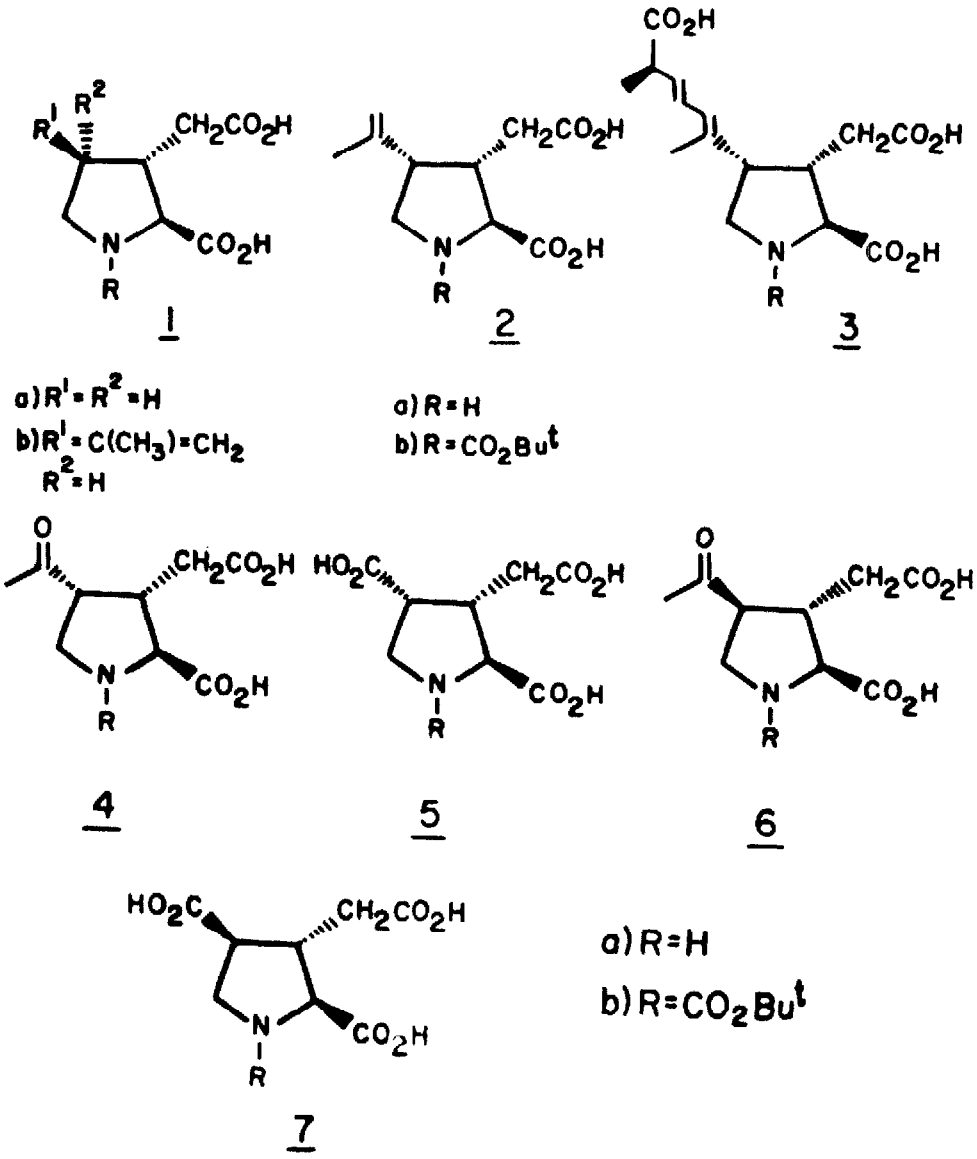
Abstract: The synthesis of the novel epimeric amino acids 5a and 7a by oxidation of methyl ketones derived from kainic acid is described. On biological testing both compounds exhibit a strong neuroexcitatory activity.

Two derivatives of (2S,3R)-2-carboxy-3-pyrrolidineacetic acid (1a), namely, kainic acid (2a)[1] and domoic acid (3),[2,3] have been identified as the active principles of the anthelmintic seaweeds *Digenea simplex* and *Chondria armata*, respectively. Both compounds have later been found to be extremely potent and long-acting neuronal excitants,[4-7] and 2a is also a powerful neurotoxin. These activities are attributed to their acting as conformationally restricted analogues of the putative neurotransmitter glutamic acid. Other compound possessing the general structure 1 have been found to display varying degrees of neuroexcitatory activity.[4,6,9] These findings suggest that new amino acids of type 1 which are derived from kainic (2a) or domoic acid (3) only by changing the residue attached at C(4), may provide compounds possessing modified neuropharmacological properties.

We now report the synthesis of two novel compounds of type 1, i.e., the epimeric tricarboxylic amino acids 5a and 7a. These compounds were prepared within the framework of a research aiming at the development of glutamatergic drugs capable of mimicking, modulating or antagonizing the effects of glutamic acid.

Kainic acid (2a) was converted into its N-tert-butyloxycarbonyl derivative 2b,[10] m.p. 149-150°C (dec., from ethyl acetate-hexane) $[\alpha]_D^{22}$ -26.4° (c=1 in MeOH) in 95% yield by treatment of its sodium salt with di-tert-butyl dicarbonate.[11] Ozonolysis of 2b in methanolic solution followed by hydrolytic work-up resulted in the quantitative formation of the methyl ketone 4b,[10] m.p. 169-170°C (dec., from ethyl acetate-hexane) $[\alpha]_D^{22}$ +10.9° (c=1 in MeOH). Oxidation of 4b with alkaline hypobromite[12] gave, after acidification, the tricarboxylic acid 5b. Treatment of crude 5b with 98% formic acid caused cleavage of the N-protecting group to afford the tricarboxylic amino acid 5a,[10,13] m.p. 238-240°C (dec. from aqueous MeOH) $[\alpha]_D^{22}$ +45.5° (c=1 in H_2O), in 70% yield (based on 4b).



The C(4)-epimer of 5a, namely, the tricarboxylic amino acid 7a, was prepared by a similar reaction sequence starting from the methyl ketone 6b which was obtained by base-catalysed epimerization of the methyl ketone 4b. Thus, treatment of 4b with 0.2 N aqueous NaOH during 24 h resulted in the formation of an approximately 6:1 mixture (by 270 MHz ^{1}H-NMR), of the desired 3,4-*trans*-methyl ketone 6b and the starting 3,4-*cis*-methyl ketone 4b, isolated by acidification and extraction with ethyl acetate. This mixture was submitted to the haloform reaction to

afford a mixture of the N-protected amino acids 7b and 5b. Removal of the tert-butyloxycarbonyl group by 98% formic acid gave a ~ 6:1 mixture (by 270 MHz ^{1}H-NMR) of the tricarboxylic amino acids 7a and 5a. Trituration of this mixture with methanol gave a soluble fraction consisting of pure 7a[10,14] in 60% overall yield from 4b, m.p. 258-260°C (dec., from water) $[\alpha]_D^{22}$ -14.0° (c=0.25 in H_2O).

The assignment of the 3,4-*cis*- and 3,4-*trans*-stereochemistry to 5a and 7a respectively rests mainly on their syntheses from the methyl ketone 4b and its C(4)-epimer 6b. The ^{1}H-NMR spectra[13,14] of these compounds, measured at 270 MHz, which are fairly well resolved and can be interpreted by an approximate first order analysis, are consistent with the assigned structures. However, due to the well-known conformational flexibility of five-membered rings,[15] the observed coupling constants between H-C(3) and H-C(4) (6.2 Hz in 5a and 8.1 Hz in 7a) do not offer an unequivocal assignment of the relative configurations. The shift of 0.2 ppm to higher field in 5a as compared to 7a, of one of the diastereotopic protons of the acetic acid side chain, may be explained by a shielding of this proton by the *cis*-oriented C(4)-carboxyl group. A similar upfield shift is found for the absorptions of both methylene protons of the 3,4-*cis*-methyl ketone 4a,[16] relative to those of the 3,4-*trans*-methyl ketone 6a.[17] Another common feature in the spectra of both epimeric pairs 5a, 7a and 4a, 6a is the appearance of H-C(4) of the *cis*-isomers at lower fields than in the *trans*-isomers ($\Delta\delta$=0.31 for 5a, 7a and 0.41 for 4a, 6a).

The amino acids 5a and 7a were found to act as strong glutamate agonists when applied in a recently developed bioassay,[9] based on the ability of glutamic acid to increase the permeability of the neuronal membrane to sodium ions. They are equally powerful in increasing Na^+ efflux rates, exhibiting a potency 2.5 times that of glutamic acid and about one third of the potency of kainic acid (2a).

It has been suggested[6] that the high activities of kainic acid (2a) and domoic acid (3) are related to the *cis*-orientation of their C(3)- and C(4)-substituents. This proposition was prompted by the diminished excitatory activity displayed by α-allokainic acid (1b).[4,6] The equipotency of the epimers 5a and 7a, as well as our finding[9] that both *cis*- and *trans*-methyl ketones 4a and 6a are almost as powerful as kainic acid (2a), indicate that the 3,4-*cis*-stereochemistry is not necessarily an essential structural feature for the activity of compounds of type 1.

We thank Mrs. R. Kuperman and Mr. N. Tal for technical assistance. This research was supported by grants from the Israel Commission for Basic Research, and from the DGRST.

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14. ^{1}H-NMR (270 MHz, D_2O) δ: 2.79/dxd, J=16.5 Hz and 7.4 Hz, 1H (one of CH_2CO_2H); 2.88-3.04 /m with a detectable splitting of 4.8 Hz, 2H [one of CH_2CO_2H and H-C(3)]; 3.17/q, J=8.1 Hz, 1H,[H-C(4)]; 3.67/d, J=8.1 Hz, 2H [H_2-C(5)]; 4.00/d, J=8.1 Hz, 1H [H-C(2)].
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(Received in UK 31 March 1980)