SYNTHESIS OF CARDIAC STEROID 3-SUBEROYLBRADYKININ ESTERS

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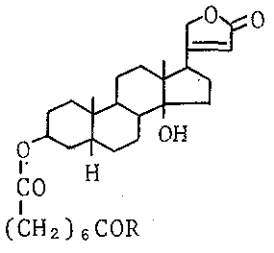
The cardiac steroid 3-suberoylbradykinin esters which may possibly occur in the toad venom, were synthesized by the activated ester method. Digitoxigenin 3-hemisuberate and bufotalin 3-hemisuberate were converted by the DCC method to the p-nitrophenyl esters, which in turn were condensed with bradykinin in aqueous pyridine to yield the desired compounds (1c, 2c).

In recent years three novel types of bufotoxins in which the succinoyl, adipoyl, and pimeloyl groups are displaced for the suberoyl residue of the so-called "bufotoxin",[1] have been isolated from the skin of Bufo vulgaris formosus Boulenger.[2] It has previously been suggested that the genuine cardiac steroid in the toad venom may be a peptide conjugate linked with L-arginyl-L-arginyl-L-proline.[3] In addition, the occurrence of the physiologically active peptides in the skin of amphibian animals has already been disclosed.[4] In these respects the physiological activity of the bradykinin-linked cardiac steroid which may possibly occur in the animal kingdom, seems to be of interest. Now the synthesis of 3-suberoylbradykinin esters of digitoxigenin and bufotalin is reported.

In a preceding paper the preparation of 3-suberoylarginine ester of digitoxigenin from its 3-hemisuberate via the p-nitrophenyl ester was described.[5] It is sufficiently substantiated that condensation reaction of the activated ester with arginylproline acetate takes place preferentially at the α-amino group rather than the guanidino residue.[5b] The p-nitrophenyl ester method, therefore, appeared to be promising for the preparation of the desired compounds.



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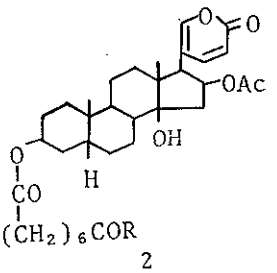
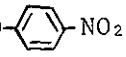


2

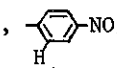
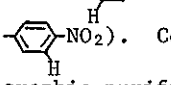
a : R=OH

b : R=O- 

c : R=Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg·OH

When 3-suberoyl-p-nitrophenyl ester of digitoxigenin (1b), derivable from the 3-hemisuberate (1a), was stirred with bradykinin (H·Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg·OH·2AcOH·$5H_2O$) in aqueous pyridine at room temperature, facile condensation was effected. Subsequent purification by column chromatography on silica gel using $CHCl_3$-MeOH-H_2O (70:30:6), followed by gel filtration on Sephadex LH-20 using MeOH gave digitoxigenin 3-suberoylbradykinin ester (1c) as colorless amorphous substance (from MeOH-ether), mp 192-195°, $[\alpha]_D^{19}$ +7.0° (c=0.14, MeOH), n.m.r. spectrum (CD_3OD);δ 0.88 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 4.96 (2H, m, 21-CH_2), 5.08 (1H, m, 3α-H), 5.90

(1H, m, 22-H), 7.24 (10H, broad s, aromatic H of phenylalanine). This compound showed a positive result with Sakaguchi's reagent and a negative test with ninhydrin indicating the formation of a peptide bond involving the α-amino group of arginine.

In similar fashion the synthesis of 3-suberoylbradykinin ester of bufotalin was also undertaken. By treatment with p-nitrophenol in the presence of N,N'-dicyclohexylcarbodiimide (DCC), bufotalin 3-hemisuberate (2a)[6] was readily transformed into the p-nitrophenyl ester (2b) as colorless prisms (from MeOH), mp 165.5-167°, $[\alpha]_D^{21}$ +10.9° (c=0.73, $CHCl_3$), n.m.r. spectrum ($CDCl_3$);δ 0.78 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.85 (3H, s, 16β-$OCOCH_3$), 5.08 (1H, m, 3α-H), 5.50 (1H, m, 16α-H), 6.16 (1H, d, J=9 Hz, 23-H), 7.20 (1H, d, J=2 Hz, 21-H), 7.24 (2H, d, J=9 Hz, ), 8.01 (1H, q, J=9,2 Hz, 22-H), 8.24 (2H, d, J=9 Hz, ). Condensation of 2b with bradykinin, followed by chromatographic purification on silica gel and Sephadex LH-20 in the manner described above gave the desired 3-suberoylbradykinin ester of bufotalin (2c) as colorless amorphous substance (from MeOH-ether), mp 183.5-185.5°, $[\alpha]_D^{21}$ -14.8° (c=0.07, MeOH), n.m.r. spectrum (CD_3OD);δ 0.77 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 1.86 (3H, s, 16β-$OCOCH_3$), 5.08 (1H, m, 3α-H), 5.48 (1H, m, 16α-H), 6.20 (1H, d, J=10 Hz, 23-H), 7.22 (10H, broad s, aromatic H of phenylalanine), 7.41 (1H, d, J=2 Hz, 21-H), 8.21 (1H, q, J=10,2 Hz, 22-H). The formation of a peptide bond involving the α-amino group of arginine was confirmed by the color tests.

The bradykinin-linked cardiac steroids structurally related to the bufotoxin will serve as useful references for the studies on the toad venom constituents. The results of the pharmacological test will be

reported elsewhere in the near future.

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REFERENCES

1 H.Wieland and R.Alles, Ber., 1922, 55, 1789; H.Wieland, G.Hesse, and R.Hüttel, Ann., 1936, 524, 203; H.Wieland and H.Behringer, ibid., 1941, 549, 209; H.O.Linde-Tempel, Helv. Chim. Acta, 1970, 53, 2188; K.Shimada, Y.Fujii, E.Mitsuishi, and T.Nambara, Chem. Ind., 1974, 342.

2 K.Shimada, Y.Fujii, E.Mitsuishi, and T.Nambara, Tetrahedron Lett., 1974, 467; K.Shimada, Y.Fujii, and T.Nambara, Chem. Ind., 1974, 963; K.Shimada, Y.Fujii, Y.Niizaki, and T.Nambara, Tetrahedron Lett., 1975, 653; Y.Fujii, K.Shimada, Y.Niizaki, and T.Nambara, ibid., 1975, 3017; K.Shimada, Y.Sato, Y.Fujii, and T.Nambara, Chem. Pharm. Bull., 1976, 24, 1118.

3 G.R.Pettit, R.L.Smith, and H.Klinger, J. Med. Chem., 1967, 10, 145.

4 V.Erspamer and A.Anastasi, 'Hypotensive Peptides,' ed. by E.G.Erdös, N.Back, and F.Sicuteri, Springer-Verlag, Inc., New York, 1966, pp 63-75.

5 a) K.Shimada, Y.Fujii, and T.Nambara, Chem. Ind., 1972, 258; b) idem. Chem. Pharm. Bull., 1973, 21, 2183.

6 N.Höriger, D.Zivanov, H.H.A.Linde, and K.Meyer, Helv. Chim. Acta, 1970, 53, 1993; G.R.Pettit and Y.Kamano, Chem. Commun., 1972, 45.

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