44. Synthesis of [2-*p*-Fluorophenylalanine]oxytocin and its Desamino Analogue Using the S-Acetamidomethyl Protecting Group

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Summary. [2-*p*-Fluorophenylalanine]oxytocin (**1a**), desamino-[2-*p*-fluorophenylalanine]oxytocin (**1b**), and desamino-oxytocin (**2**) have been synthesised *via* intermediates containing S-acetamidomethyl-cysteine. The protected linear peptides were built up using both stepwise and fragment-condensation procedures, and the S-protecting groups were removed by iodine with simultaneous formation of the disulfide bridge. The uterotonic activities *in vitro* of the analogues have been determined. The close similarity of the ^{19}F-NMR. spectra indicates that the *p*-fluorobenzyl side chain is freely exposed to the solvent in the disulfide-bridged hormone analogues as well as in their S-protected, acyclic precursors.

p-Fluorophenylalanine analogues of biologically active peptides have been prepared for the study of structure-activity relationships [1] and, more recently, for conformational studies by ^{19}F-NMR. spectroscopy [2]. An early attempt [3] to prepare the [2-*p*-fluorophenylalanine] analogue of oxytocin failed as removal of the S-benzyl protecting groups with sodium in liquid ammonia also involved loss of halogen [4]. In this paper we describe a new synthetic approach for [2-*p*-fluorophenylalanine]oxytocin (**1a**) and its desamino analogue (**1b**).

X-Phe(F)-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2 β-Mp-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

1a: X = Cys, **1b**: X = Mp **2**

The cleavage of S-benzyl protecting groups with hydrogen fluoride [5] should, in principle, be suitable for the preparation of halogenated oxytocin analogues and has been used very recently for the synthesis of [2-pentafluorophenylalanine]oxytocin [6]. However, we decided that the use of a sulfur-protecting group other than benzyl would be more generally useful for this and other purposes.

Although several S-protecting groups have been tested in syntheses of oxytocin (trityl [7], *p*-methoxybenzyl [5] [8], ethylcarbamoyl [9], benzyloxycarbonyl [10–11], benzoyl [10], ethylmercapto [12], 2-(N-methylbenzyloxycarbamido)ethylcarbamoyl [13]) none of these seems to have been used for the preparation of analogues. The S-acetamidomethyl (Acm)[2]) protecting group is readily introduced into cysteine [16] (see also below), confers good solubility on its derivatives, and is readily removed

[1]) From the Doctoral Dissertation to be submitted by *P. Marbach.*

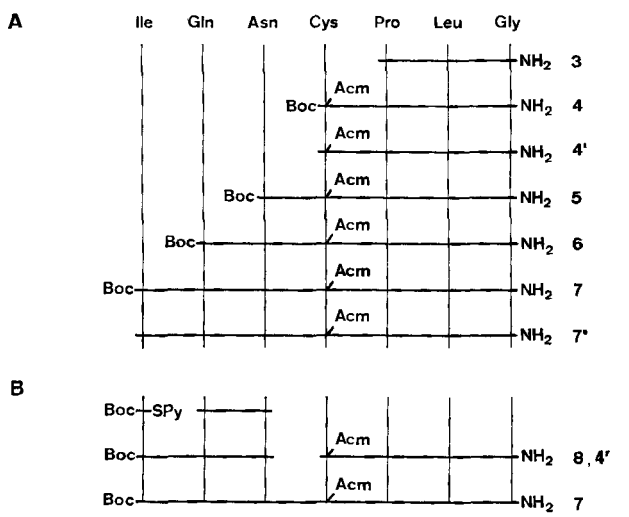
[2]) In addition to the standard abbreviations for amino acids and protecting groups [14], the following are used: Phe(F) for *p*-fluorophenylalanine, βMp for 3-mercaptopropionyl, and SPy for 2-pyridylthio; further, DCCI is used for dicyclohexylcarbodiimide, DCHA for dicyclohexylamine, DMF for dimethylformamide, HOBt for 1-hydroxybenzotriazole, NMM for N-methylmorpholine, and TMG for N,N,N′,N′-tetramethylguanidine. All amino acids (except glycine) are of the L configuration unless otherwise stated. The naming of analogues accords with current convention [15].

by iodine with simultaneous formation of disulfide bonds [17]. Its utility for our purpose was verified by the preparation of crystalline desamino-oxytocin (**2**).

S-Acetamidomethyl-cysteine has been obtained by reaction of cysteine with acetamidomethanol in aqueous acid solution or in anhydrous hydrogen fluoride [16]. The first of these methods leads to by-products, the second is hardly suitable for large-scale use. By analogy with the procedure for introducing S-diphenylmethyl substituents [18] the use of anhydrous trifluoroacetic acid as solvent and acid catalyst[3]) was found to be effective and convenient. 3-(Acetamidomethylthio)-propionic acid, the 'desamino' analogue of S-acetamidomethyl-cysteine, has been obtained similarly.

t-Butyloxycarbonyl-S-acetamidomethyl-cysteine [16] was coupled with the known [21] tripeptide amide **3** using dicyclohexylcarbodiimide in the presence of 1-hydroxy-benzotriazole [22] as condensing agent. The product **4** was converted to the tetrapeptide amide **4'** (isolated as the hydrochloride) by removal of the N-protecting group with hydrogen chloride in ethyl acetate. The protected heptapeptide amide **7** with the carboxyl-terminal sequence of oxytocin, the key intermediate common to the synthesis of all three analogues (**1a**, **1b**, and **2**), was prepared from the tetrapeptide amide **4'** by two routes (Scheme 1, A and B) based on stepwise chain extension and on fragment condensation, respectively.

Scheme 1



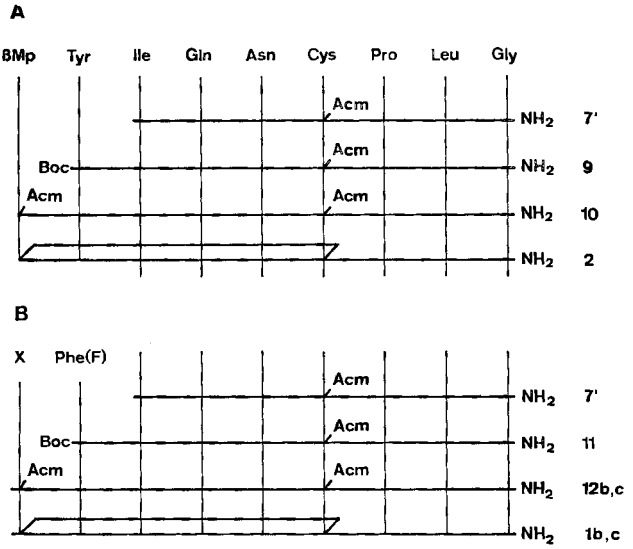
In the first approach, the Boc derivatives of asparagine, glutamine, and isoleucine were used for successive couplings by dicyclohexylcarbodiimide and 1-hydroxy-benzotriazole; hydrogen chloride was used for removal of the N-protecting groups. The protected pentapeptide **5** proved to be so water-soluble that its isolation by the

[3]) This possibility was discussed with Dr. *D. F. Veber*, of *Merck Sharp & Dohme* Research Laboratories, in 1970. Since completion of our work, new methods for the purification [19] and preparation [20] of S-acetamidomethyl-cysteine have been described.

conventional solvent extraction work-up presented difficulties; it could, however, be isolated (see [23]) by passage of the reaction mixture through a mixed bed of the macroreticular ion exchange resins, *Amberlysts* 15 and A-21, effective in organic solvents. The column retained the chloride ions originating from the peptide amide hydrochloride, the tertiary amine added to neutralise the hydrogen chloride, any excess of the carboxyl or amino component still present, and the hydroxybenzotriazole, so that the protected peptide **5** recovered from the eluate was only contaminated with a small amount of dicyclohexylurea, removable by recrystallisation. This procedure proved so effective and convenient that it was applied in most subsequent steps[4]).

As regards the fragment condensation approach, *Jošt* [26] has already pointed out the utility of suitably substituted peptides corresponding to sequences 2–5 or 3–5 of oxytocin for the preparation of analogues using azide coupling. *König & Geiger* [22] have furnished an alternative racemisation-free coupling method applicable to carboxyl-terminal asparagine. The protected tripeptide **8** requisite for this synthesis of **7** was prepared by acylating glutaminyl-asparagine [27] as the tetramethylguanidine salt [28] with the 2-pyridyl thiolester of Boc-isoleucine, in dimethylformamide (DMF) [29] or in aqueous dioxan. We have found that 2-mercaptopyridine esters are relatively resistant to aqueous base hydrolysis – as, indeed, would be expected from their character as thiol esters [30] and the mechanism of activation by intramolecular general base catalysis [31] – and therefore particularly suitable for use in aqueous solvents [32].

Scheme 2



b: X = βMp, c: X = Boc-Cys

[4]) A macroreticular ion exchange resin was first used in peptide chemistry by *Young* and his coworkers [24] for the isolation of 4-picolyl esters. A recent paper describes the use of conventional ion exchange resins for the isolation of neutral protected peptides [25] in a procedure similar to ours [23]; we believe that macroreticular resins will prove to be superior.

The synthesis of the protected intermediate **10** containing the sequence of desamino-oxytocin was completed by the stepwise addition of tyrosine and 3-(acetamidomethylthio)-propionic acid to the heptapeptide amide (**7'**) obtained from **7** (Scheme 2, A). Similarly, chain extension with *p*-fluorophenylalanine followed by Boc-S-acetamidomethyl-cysteine or 3-(acetamidomethylthio)-propionic acid afforded the protected *p*-fluorophenylalanine peptides **12b** and **12c** (Scheme 2, B). The requisite *p*-fluorophenylalanine was conveniently obtained from the racemic ethyl ester by stereospecific hydrolysis with chymotrypsin [33] and was converted to the Boc derivative (isolated as the dicyclohexylamine salt) by treatment of the tetramethylguanidine salt with *t*-butyl azidoformate in dimethylformamide [16] [34].

Removal of the S-Acm protecting groups and closure of the disulfide bridge was smoothly effected with iodine in aqueous methanol by *Kamber*'s procedure [17], except that iodine was slowly added to a 0.2 mM solution of the peptide (rather than peptide to iodine [17]), in order to reduce the danger of overreaction. The products were isolated by countercurrent distribution in about 60% yields. Finally, cleavage of the N-protecting group from the Boc derivative **1c** was effected with 80% trifluoroacetic acid and the product was purified on Sephadex G-15 [35]. The acyclic peptide **13** needed for the NMR. studies was similarly obtained from **12c**. The desamino-oxytocin (**2**) crystallised readily [36] but so for the *p*-fluorophenylalanine analogue **1b** has not. The products **1a**, **1b**, and **2** were characterised by amino-acid analysis, elemental analysis, optical rotation, thin-layer chromatography (tlc.) and assay for uterotonic activity.

Cys(Acm)-Phe(F)-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH_2 · TFA **13**

[2-*p*-Fluorophenylalanine]oxytocin (**1a**) and the desamino analogue **1b** were found to have uterotonic potencies of 97.0 ± 14 and 57.3 ± 3.7 IU/μmol These values are about 2–3 times higher than those for the corresponding 2-phenylalanine peptides (32 and ~25 IU/μmol, [37–38]). That of [2-*p*-fluorophenylalanine]oxytoxin is the highest yet recorded for an oxytocin derivative modified in sequence position 2. The peptides **1a** and **1b** resemble the [2-phenylalanine] analogues (and the [4-threonine] analogues; *cf.* [39]) in that the oxytocin analogue is more active than its desamino derivative; in most other such pairs the reverse holds (see [40]).

Desamino-[2-*p*-fluorophenylalanine]oxytocin (**1b**) behaved as a partial agonist under the assay conditions used [41] (*cf.* Exp.), eliciting a maximal response of *ca.* 60–80% of that to oxytocin in different experiments. This is not unexpected since oxytocin analogues modified in position 2 generally tend to act as partial agonists or inhibitors (see [42]).

It has been proposed, on the basis of structure-activity relationships [43], that in the 'active' conformation of oxytocin, *i.e.* the conformation (or range of conformations) in which the hormone interacts with its biological receptor sites, the tyrosyl side-chain may be turned 'inward' to lie over the cyclic portion of the molecule, rather than being freely exposed to the solvent. From the evidence of thin-film dialysis and CD. measurements it has been suggested that a similar conformation may also exist in aqueous solutions [44]. Since the NMR. properties of aromatic fluoro substituents are known to be sensitive to conformational and environmental effects (see *e.g.* [45]) the ^{19}F-NMR. spectra of the disulfide-bridged oxytocin analogues

1a and **1b** were compared with the spectra of the acyclic, S-substituted precursors to see if any evidence of such a conformation could be detected. The spectra of the amino derivatives **1a** and **13** were measured in deuterium oxide containing 20% acetic acid-d_4, those of the desamino peptides **1b** and **12b** in dimethyl sulfoxide-d_6[5]). In each case, the ^{19}F-NMR. spectra of the cyclic and the acyclic derivatives were practically identical in pattern (multiplicity, peak widths, coupling constants) (Fig.)

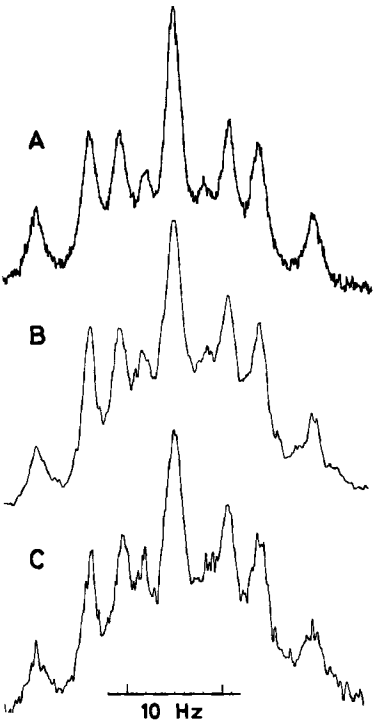


Fig. *^{19}F-NMR Spectra of Acetyl-*p*-fluorophenyl-*DL*-alanine (A), the S-Protected Acyclic Peptide* **12b** *(B), and the Oxytocin Analogue* **1b** *(C) at 94 MHz in Dimethylsulphoxide-d_6 at 31°.* Trifluoroethanol as internal reference, 0 ppm. A: 200 mM, single scan, centred at 39.08 ppm; B: 30 mM, 37 scans, centred at 39.38 ppm; C: 30 mM, 45 scans, centred at 39.28 ppm.

though they differed very slightly (5–10 Hz) in position. The spectra in each solvent were, moreover, very similar to those of N-acetyl-*p*-fluorophenyl-DL-alanine in the same solvent. We conclude that no change in the conformational freedom or environment of the fluorophenyl group takes place on formation of the disulfide ring and that it is probably exposed to solvent in both the cyclic and the acyclic derivatives. This may not, of course, apply to the tyrosine side-chain of oxytocin which might be conformationally restricted, perhaps by virtue of its hydrogen-bonding capacity. However, the rather high potency of the [2-*p*-fluorophenylalanine] analogues indi-

[5]) We are grateful to Prof. *K. Wüthrich*, Mr. *J.-P. Meraldi* and Mr. *A. Masson* for the interpretation of ^{19}F-NMR. spectra, and to the two latter for their measurement.

cates that this particular conformational feature *in solution* is not of major importance for achieving the 'active' conformation required for biological activity.

Experimental Part

General: M.p.'s (uncorrected) were determined with a capillary m.p. apparatus (*Büchi*, Flawil, Switzerland). Optical rotations, estimated to be correct to $\pm 1°$, were measured at 25°, in a 10-cm tube with a *Perkin-Elmer* 141 photoelectric polarimeter.

Materials: Most solvents and reagents were obtained from *Fluka* AG, Buchs, Switzerland. The *Amberlyst* ion exchange resins (*Rohm & Haas*, Philadelphia, USA) and *Sephadex* cross-linked dextran gels (*Pharmacia* AB, Uppsala, Sweden) were prepared for use according to the manufacturers' instructions.

Thin-layer chromatography (tlc.) and *electrophoresis*: Thin-layer chromatograms were run on silica gel (thickness 0.25 mm; DC-Fertigplatten F-254, *Merck*, Darmstadt, Germany) in the solvent systems (composition by volume) A: *n*-BuOH/H_2O/AcOH 4:1:1; B: MeOH/$CHCl_3$ 1:1; C: 2-propanol/pyridine/H_2O 7:6:6 (the Rf values given are to be regarded as a general indication of chromatographic behaviour, not as physical constants). Electrophoresis was carried out in a moist-chamber apparatus (*Camag*, Muttenz, Switzerland) on cellulose-coated (0.1 mm thickness) plates (*Macherey-Nagel*, Düren, Switzerland) at a potential gradient of about 25 V/cm, during 45–60 min, using the electrolytes (composition by volume) D: 88% HCOOH/AcOH/H_2O 15:10:75, pH 1.9, and E: pyridine/AcOH/H_2O 1:10:90, pH 3.6. Mobilities are expressed as m_{Arg}, the ratio of the distance from the origin for the sample and the reference arginine. Detection was carried out with ninhydrin [46], by a modification [47] of the procedure of *Reindel & Hoppe* [48], with the *Pauly* reagent [49], with iodine vapour, and by observing fluorescence quenching on the plates, as appropriate. In testing for purity, samples were applied in 50–150 μg amounts.

Analytical: Samples for elemental analysis were dried for 10–15 h at 40–60°/0.1 Torr; water was determined with the *Karl Fischer* reagent. Where, on the basis of elemental analysis, compounds are formulated as containing solvent this does not necessarily imply that they are defined solvates; particularly in the case of amorphous products, solvent retention may have been due to mild drying conditions. For amino-acid analysis samples were hydrolysed for 20 h with 6M HCl containing a trace of phenol in evacuated tubes at 110°. A *Beckman* 120B amino-acid analyser was used; ratios are referred to Gly. Phe(F) emerged at the position of Phe and was assumed to have a colour constant 1% lower [2].

Spectroscopy: The ^{19}F-NMR. spectra were measured with a *Varian* XL-100 spectrometer at 94 MHz, using a CAT programme (50–90 scans), in 5-mm o. d. sample tubes at 31°; line positions are relative to trifluoroethanol. The ^{1}H-NMR. spectra were recorded with a *Varian* T-60 spectrometer at 60 MHz and 30° with *ca.* 0.5M solutions, using tetramethylsilane (TMS) as internal reference.

Standard preparative procedures: Peptide amide hydrochlorides were prepared from the Boc derivatives by treatment for 30 min with a saturated solution of HCl in EtOAc at room temperature, and isolated by dilution with an excess of dry ether, washing with ether, and thorough drying over NaOH *in vacuo*. Immediately before coupling, they were treated with $NaHCO_3$ in DMF [23] to remove any excess HCl.

Couplings with dicyclohexylcarbodiimide (DCCI) and 1-hydroxybenzotriazole (HOBt) were carried out by treating the amino component hydrochloride (1 mol), the carboxyl component (1–1.2 mol), N-methylmorpholine (NMM, 1 mol), and HOBt (1.2–1.5 mol) in DMF with DCCI (1.1–1.3 mol) at $-5°$ with stirring, which was continued whilst the mixture came to room temperature (1–3 h) and was kept there until the amino component could no longer be detected by tlc. (5–15 h). The solution was diluted with an equal volume of water, filtered, and worked up: a) If water precipitated the product, this was washed with water, 2-propanol, and ether, digested with 2-propanol to remove dicyclohexylurea, and recrystallised from a suitable solvent. b) If the product was soluble in water/DMF its solution was passed through a column containing *Amberlysts* 15 and A-21 prepared as below and equilibrated with 50% aq. DMF. The column was washed with water and the combined eluates were evaporated to small volume or to dryness.

Mixed-bed deionisation (general procedure). The sulfonic acid resin *Amberlyst* 15 (H^+ form) and the weakly basic *Amberlyst* A-21 (free base) are stored under MeOH. A column is filled with equal settled volumes of the two resins previously mixed and dried on a rotary evaporator at 50°, the quantities being chosen so as to give a *ca.* 10-fold excess of *Amberlyst* A-21 with respect to the amount of HOBt in the reaction mixture (about 10 ml settled volume of each resin for 1 mmol). After the column has been slowly filled with the appropriate solvent by upward flow and washed with about 5 column volumes of the solvent by downward (gravity) flow, the reaction mixture, in the same solvent, is introduced at the top and the column is washed with the same or a compatible solvent (flow rate about 4–6 bed volumes/h). The product emerges with the break-through volume.

Removal of Acm protecting groups with formation of the disulfide bridge was effected by addition of I_2 (2.5 mol/Acm group) in MeOH, over 60–75 min, to a vigorously stirred *ca.* 0.2 mM solution of the protected peptide in 75% (v/v) aqueous methanol. The mixture, kept at room temperature until no more starting material was detected by tlc. in solvent system A (3–5 h), was evaporated down to 15–20 ml, the excess I_2 distilling with the solvents to give a colourless solution. This solution, acidified with a drop of AcOH and filtered through a small (1 × 5 cm) column of Sephadex G-10 to remove residual traces of I_2 if necessary, was directly equilibrated with the upper phase (20 ml) of the solvent system chosen for counter-current distribution (CCD.), which was carried out in a 'Steady State Distribution Apparatus' (*Quickfit & Quartz*, Stone, Staffs., England). The separation was monitored by tlc. (detection by *Reindel-Hoppe* reagents). On completion of the run the contents of the appropriate tubes were pooled, evaporated to small volume, and lyophilised.

Evaporations were carried out in a rotary evaporator (*Büchi*, Flawil, Switzerland) at a temperature not exceeding 40°; solutions in DMF were evaporated at 0.5 Torr.

Bio-assay. Using *van Dyke-Hastings* medium (without Mg [41]) and isometric recording, and organs from virgin rats in natural oestrus or oestrus to metoestrus, *uterotonic activity* was assayed [50] against the 'Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances'. Maximal responses were determined by a cumulative dose procedure [51]. For **1b**, the potency was calculated from responses in the lower range of the dose-response curves since the maximal response differed from that to oxytocin.

Syntheses: Unless otherwise indicated standard procedures were used. – *Cys(Acm). HCl.* Cysteine hydrochloride (1.576 g; 10 mmol) and N-hydroxymethylacetamide [52] (0.890 g; 10 mmol) in trifluoroacetic acid (10 ml) were stirred at room temperature for 30 min and evaporated to dryness; the residue was dissolved in 1M HCl, taken to dryness, and this was repeated to give a residue which after recrystallisation from 2-propanol, washing with ether, and drying, yielded 1.62 g (71%); m.p. 155–157° (dec.), 166–168° (dec.) after recrystallisation from aq. 2-propanol; $[\alpha]_D = -33.2°$ ($c = 1$, H_2O). M.p. 159–163° (dec.), $[\alpha]_{589}^{25} = -30.7°$ ($c = 1$, H_2O) [16].

βMp (Acm) was prepared from 3-mercaptopropionic acid as described for the cysteine derivative above (but evaporations with HCl omitted). Yield, 63%, m.p. 74°; ^{1}H-NMR. spectrum: δ (Me_2SO-d_6) 1.87 (*s*, $-CH_3$); 2.37–2.93 (A_2B_2, $-CH_2-CH_2-$, overlapping with solvent peaks); 4.27 (*d*, $-CH_2-$); 8.47 (*s*, $-NH-$); after addition of about 20% D_2O the *s* at 8.47 ppm disappeared and *d* at 4.27 ppm collapsed to *s*.

$C_6H_{11}NO_3S$ (177.2) Calc. C 40.67 H 6.26 N 7.90% Found C 40.62 H 6.40 N 7.74%

Boc-Cys(Acm)-Pro-Leu-Gly-NH$_2$ (**4**). A solution of Pro-Leu-Gly-NH_2 · $\frac{1}{2}H_2O$ (**3**) [21] (2.93 g), Boc-Cys(Acm) (2.91 g), and HOBt (1.5 g) in DMF (10 ml) was treated with DCCI (2.4 g), see above. After 15 h the solution, was diluted with water, filtered, and evaporated to an oily residue which crystallised from 2-propanol as the hemihydrate; yield 4.94 g (87%), m.p. 115–118°, unchanged by recrystallisation; $[\alpha]_D = -92.5°$ ($c = 1$, H_2O), Rf 0.54 (A), 0.75 (B).

$C_{24}H_{42}N_6O_7S \cdot \frac{1}{2}H_2O$	Calc.	C 50.78	H 7.63	N 14.80	H_2O 1.59%
(567.7)	Found	,, 50.58	,, 7.65	,, 14.59	,, 1.38%

Boc-Asn-Cys(Acm)-Pro-Leu-Gly-NH$_2$ (**5**). The hydrochloride **4'** (3.46 g) [from **4** in 90% yield; Rf 0.22 (A), 0.32 (B), m_{Arg} 0.5 (D), 0.7 (E)], Boc-Asn (1.70 g), NMM (0.708 g), and HOBt (1.35 g) in DMF (10 ml) were treated with DCCI (1.6 g) as above. After 4 h the solution was diluted

with water, filtered, and deionised. The eluates, evaporated to small volume, gave a gel on addition of 2-propanol. Yield 68%, melting from 171° (dec.), $[\alpha]_D = -63.5°$ ($c = 1.5$, DMF), Rf 0.45 (A), 0.65 (B).

$C_{28}H_{48}N_8O_9S \cdot H_2O$	Calc.	C 49.99	H 7.19	N 16.65	H_2O 2.61%
(781.8)	Found	,, 49.96	,, 7.22	,, 16.32	,, 2.68%

Boc-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH$_2$ (**6**). The hydrochloride (2.4 g) prepared from **5** [97% yield; Rf 0.19 (A), 0.25 (B), m_{Arg} 0.5 (D), 0.72 (E)], Boc-Gln (1.0 g), NMM (0.444 ml), and HOBt (0.800 g) in DMF (8 ml) were treated with DCCI (0.940 g) for 5 h; after the standard procedure, the mixed-bed eluate was evaporated to *ca.* 1 ml. Dilution with 2-propanol and treatment with di-2-propyl ether until cloudy, gave a gelatinous product on cooling to 0°; yield 2.31 g (70%), apparently a sesquihydrate; melting from 175° (dec.), $[\alpha]_D = -60.3°$ ($c = 1.7$, DMF); Rf 0.35 (A), 0.5 (B).

$C_{33}H_{56}N_{10}O_{11}S \cdot 1\frac{1}{2}H_2O$	Calc.	C 47.87	H 7.18	N 16.92%	
(827.9)	Found	,, 47.83	,, 7.23	,, 16.54%	

Boc-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH$_2$ (**7**). *a*) The hexapeptide hydrochloride (1.50 g) obtained from **6** [94% yield, Rf 0.11 (A), 0.05 (B), m_{Arg} 0.35 (D), 0.58 (E)], Boc-Ile. $\frac{1}{2}H_2O$ (0.500 g), NMM (0.230 ml), and HOBt (0.30 g) in DMF (8 ml) were treated with DCCI (0.47 g). After 15 h the reaction mixture had set to a gel; it was diluted with DMF (10 ml) and treated with Boc-Ile · $\frac{1}{2}H_2O$ (110 mg) preactivated with HOBt (75 mg) and DCCI (110 mg) in DMF (5 ml) during 45 min at 0°. After 2 h the mixture was diluted with water until filtrable, filtered, evaporated to half volume, deionised, and taken to dryness. The residue was crystallised from EtOH; yield 1.51 g (77%) of the EtOH solvate melting from 214° (dec.), $[\alpha]_D = -54.0°$ ($c = 1$, DMF), Rf 0.37 (A), 0.58 (B).

$C_{39}H_{67}N_{11}O_{12}S \cdot C_2H_6O$	Calc.	C 51.29	H 7.66	N 16.05%	
(960.2)	Found	,, 51.15	,, 7.66	,, 15.79%	

b) The tetrapeptide hydrochloride **4'** (1.98 g), the Boc-tripeptide **8** (1.89 g), NMM (0.444 ml), and HOBt (0.675 g) in DMF (40 ml) were coupled with DCCI (0.900 g) under the standard conditions. After 20 h, work-up as under *a*) yielded 2.50 g (65%) after crystallisation from aq. EtOH, identical by melting behaviour, optical rotation, and Rf values with product prepared as under *a*) (**7**).

Boc-Ile-Gln-Asn (**8**). *a*) A solution of Gln-Asn [27] (7.8 g; 30 mmol) in water (50 ml) containing TMG (3.45 g; 30 mmol) was lyophilised, the residue was taken up in DMF (100 ml) and treated with Boc-Ile-SPy (10.7 g; 33 mmol) in DMF (20 ml). After 5 h, evaporation to dryness gave a residue which after crystallisation from, and washing with ether until practically colourless, was dissolved in water at 40°. The solution was brought to pH 3 by gradual addition of AcOH, which caused progressive crystallisation. The product was suspended in boiling aq. MeOH, cooled, filtered, and washed with acetone and ether; yield 9.75 g (72%), melting from 217° (dec.), $[\alpha]_D = -43.6°$ ($c = 2$, 0.1M NaOH), Rf 0.39 (A), 0.13 (B).

$C_{20}H_{35}N_5O_8$	Calc.	C 50.73	H 7.45	N 14.79%	
(473.5)	Found	,, 50.91	,, 7.43	,, 14.62%	(corr. for H_2O 0.76%)

b) Gln-Asn (440 mg) and TMG (194 mg) in water (3 ml) were treated at 0° with Boc-Ile-SPy (550 mg) in dioxan (5 ml). After stirring overnight at room temperature the dioxan was evaporated, the solution was brought to pH 3 with AcOH, and extracted with ether (4 × 5 ml). The product which gradually crystallised from the aqueous solution was isolated as under *a*); yield 400 mg (51%), melting from 215° (dec.), $[\alpha]_D = -43.4°$ ($c = 2$, 0.1M NaOH), identical in chromatographic behaviour with product prepared as under *a*).

Boc-Tyr-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH$_2$ (**9**). The heptapeptide hydrochloride **7'** (170 mg) [from **7** in 93% yield; Rf 0.17 (A), 0.15 (B), m_{Arg} 0.30 (D), 0.54 (E)], Boc-Tyr · DCHA[2]) (96 mg), and HOBt (40 mg) in DMF (5 ml) were treated with DCCI (45 mg). After 15 h the mixture was treated as usual and the product was isolated after deionisation. Crystallisation from aq. MeOH and washing with 2-propanol and ether afforded 150 mg (70%) of a hemihydrate, melting from 205° (dec.), $[\alpha]_D = -45.2°$ ($c = 2.2$, DMF), Rf 0.40 (A), 0.64 (B).

$C_{48}H_{76}N_{12}O_{14}S \cdot \frac{1}{2}H_2O$	Calc.	C 53.07	H 7.14	N 15.47	H_2O 0.83%	
(1086.3)	Found	,, 52.84	,, 7.30	,, 15.23	,, 0.99%	

Boc-Phe(F) · DCHA. Phe(F) [33] [53] (366 mg; 2 mmol) and TMG[2]) (460 mg; 4 mmol) in DMF (5 ml) were treated at 0° with *t*-butyl azidoformate (315 mg; ~2.2 mmol). After standing overnight at room temperature the solution was diluted with water, washed with EtOAc (2 × 5 ml), acidified to pH 2 with 1M H_2SO_4, and extracted with EtOAc. The extract was washed with saturated NaCl, dried over $MgSO_4$, evaporated to about 10 ml and treated with DCHA (360 mg; 2 mmol). The crystalline product was thoroughly washed with ether; yield 800 mg (86%), m.p. 190°, $[\alpha]_D = +30.0°$ (c = 0.9, DMF), Rf 0.66 (A), 0.52 (B).

$C_{26}H_{41}FN_2O_4$ (464.6) Calc. C 67.21 H 8.89 N 6.03% Found C 67.25 H 9.00 N 5.69%

βMp(Acm)-Tyr-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH₂ (**10**). The octapeptide hydrochloride (142 mg) from **9** [95% yield; Rf 0.21 (A), 0.10 (B), m_{Arg} 0.26 (D), 0.50 (E)], βMp(Acm) (30 mg), NMM (15 µl), and HOBt (27 mg) in DMF (3 ml) were treated with DCCI (39 mg). After standing overnight, dilution with water, and mixed-bed deionisation the eluate, evaporated to small volume, gave a precipitate with 2-propanol; yield 114 mg of a hemihydrate, melting from 230° (dec.), $[\alpha]_D = -48.9°$ (c = 0.48, DMF), Rf 0.25 (A), 0.50 (B).

$C_{49}H_{77}N_{13}O_{14}S_2 \cdot \frac{1}{2}H_2O$ (1145.4) Calc. C 51.38 H 6.86 N 15.90 H_2O 0.79%
Found ,, 51.18 ,, 6.31 ,, 15.69 ,, 0.97%

Boc-Phe(F)-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH₂ (**11**). The heptapeptide hydrochloride **7'** (424 mg), Boc-Phe(F) · DCHA (290 mg), and HOBt (100 mg) in DMF (5 ml) were treated with DCCI (112 mg). After standing overnight, dilution with an equal volume of water, and cooling to 0°, filtration gave a residue which was washed with water, acetone, and ether, digested with 2-propanol (30 ml) at 50°, cooled to room temperature, and washed with ether to give a 2-propanol solvate: yield 390 mg (72%), melting from 225° (dec.), $[\alpha]_D = -35.0°$ (c = 0.6, DMF), Rf 0.30 (A), 0.66 (B).

$C_{48}H_{75}FN_{12}O_{13} \cdot C_3H_8O$ (1139.4) Calc. C 53.70 H 7.08 N 14.75%
Found ,, 53.63 ,, 7.05 ,, 14.71% (corr. for H_2O 0.45%)

βMp(Acm)-Phe(F)-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH₂ (**12b**). The octapeptide hydrochloride (235 mg) prepared from **11** [92% yield; Rf 0.34 (A), 0.54 (B), m_{Arg} 0.26 (D), 0.47 (E)], βMp(Acm) (55 mg), NMM (30 µl) and HOBt (55 mg) in DMF (3 ml) treated with DCCI (70 mg) gave on standing overnight a gelatinous mixture which was diluted with water and centrifuged. The precipitate was repeatedly washed with water, 2-propanol, and ether, with centrifugation. The residue (190 mg; 72%) was extracted with hot 2-propanol and dried. Melting from 210° (dec.), $[\alpha]_D = -72.0°$ (c = 0.58, AcOH), Rf 0.30 (A), 0.54 (B). Analysis fitted a solvate with 1 mol H_2O and ½ mol 2-propanol.

$C_{49}H_{77}FN_{13}O_{13}S_2 \cdot \frac{1}{2}C_3H_8O \cdot H_2O$ (1186.4) Calc. C 51.12 H 6.97 N 15.35 H_2O 1.52%
Found ,, 50.92 ,, 6.84 ,, 15.28 ,, 1.24%

Boc-Cys(Acm)-Phe(F)-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH₂ (**12c**). The octapeptide hydrochloride (101.5 mg) from **11** as above, Boc-Cys(Acm) (44 mg), NMM (11 µl), and HOBt (27 mg) in DMF (3 ml) were treated with DCCI (33 mg) and the product was isolated as described for **12b**; yield 90 mg (72%), melting from 237° (dec.), $[\alpha]_D = -51.5°$ (c = 0.5, DMF), Rf 0.40 (A), 0.58 (B).

$C_{54}H_{85}FN_{14}O_{15}S_2 \cdot 1\frac{1}{2}H_2O$ (1280.5) Calc. C 50.65 H 6.93 N 15.31%
Found ,, 50.66 ,, 6.57 ,, 15.35%

Cys(Acm)-Phe(F)-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH₂ · CF₃COOH (**13**). The protected nonapeptide **12c** (56 mg) was treated with 80% aq. trifluoroacetic acid (2 ml) at room temperature for 30 min and the solution was evaporated to dryness. The residue was recrystallised from water, washed with 2-propanol and ether, and dried over NaOH for 24 h at 20°/15 Torr. Yield 32 mg (60%), decomposing from 190°, $[\alpha]_D = -52.8°$ (c = 0.47, 80% aq. DMF), Rf 0.19 (A), m_{Arg} 0.43 (E).

$C_{49}H_{77}FN_{14}O_{15}S_2 \cdot C_2HF_3 \cdot 2H_2O$ (1303.4) Calc. C 47.00 H 6.34 N 15.04%
Found ,, 46.83 ,, 6.48 ,, 14.88%

The number and character of the fluorine atoms was established by ^{19}F-NMR.

Desamino-oxytocin (**2**). The S-protected peptide **10** (80 mg) in 75% aq. MeOH (400 ml) was treated with I_2 (45 mg) in MeOH (100 ml) during 1 h and after 4 h standing, the solution was evaporated to 20 ml (bath temperature 30°), filtered through *Sephadex* G-10, and equilibrated with

the upper phase of the solvent system *n*-BuOH/toluene/0.05% aq. AcOH (3:2:5) [54]. After CCD. through 178 transfers of upper and 22 of lower phase the product ($K = 0.5$; *cf.* [54]) was isolated from tubes 30–60; yield 39 mg (56%). Crystallisation from water (1.5 ml) containing a trace of AcOH afforded 24 mg (34%) of the trihydrate (*cf.* [23]), m.p. 174–175°, $[\alpha]_D = -90.0°$ ($c = 0.48$, 1M AcOH), Rf 0.35 (A), 0.43 (B), uterotonic activity 599 ± 25 IU/mg. Amino-acid ratios: Asp 1.02 (1), Glu 1.08 (1), Pro 1.01 (1), Gly 1, ½Cys_2 1.08 (1), Ile 0.92 (1), Leu 0.99 (1), Tyr 0.96 (1).

$C_{43}H_{65}N_{11}O_{12}S_2 \cdot 3H_2O$	Calc.	C 49.37	H 6.84	N 14.73	H_2O 5.17%
(1046.2)	Found	,, 49.02	,, 6.74	,, 14.44	,, 5.50%

The literature records m.p. 179°, $[\alpha]_D^{20} = -90.4°$ [36] or m.p. 174–176°, $[\alpha]_D^{25} = -92.4°$ ($c = 0.5$, 1M AcOH) [23] and a uterotonic activity of 551 ± 17 IU/mg (oestrus); 899 ± 24 IU/mg (metoestrus) [55], or 698 ± 58 IU/mg (oestrus or oestrus to metoestrus) [23].

*Desamino-[2-*p*-fluorophenylalanine]oxytocin* (**1b**). The protected peptide **12b** (80 mg) was treated with I_2 and after 3 h the mixture was worked up as for **2** above. After CCD. (100 transfers of upper and 20 of lower phase) the product ($K = 3.3$) was isolated from tubes 60–86. The lyophilisate, dried for 10 h at 40° and 0.1 Torr, yielded 47 mg (67%), $[\alpha]_D = -103.4°$ ($c = 0.62$, 1M AcOH), Rf 0.40 (A), 0.45 (B), uterotonic activity 57.3 ± 3.7 IU/μmol. Amino-acid ratios: Asp 0.97 (1), Glu 1.01 (1), Pro 1.01 (1), Gly 1, ½Cys_2 1.16 (1), Ile 0.99 (1), Leu 1.01 (1), Phe(F) 0.99 (1).

$C_{43}H_{64}FN_{11}O_{11}S_2 \cdot 4H_2O$	Calc.	C 48.43	H 6.81	N 14.45	H_2O 6.76%
(1066.2)	Found	,, 48.74	,, 6.81	,, 14.48	,, 7.07%

Presence of F was confirmed by ^{19}F-NMR spectra.

*[2-*p*-Fluorophenylalanine]oxytocin* (**1a**). The protected nonapeptide **12a** (50 mg) in 75% aq. MeOH (250 ml) was treated with I_2 (30 mg) in MeOH (40 ml) for 5 h, the solution was evaporated to 20 ml and equilibrated with 20 ml of the upper phase of the solvent system *n*-BuOH/benzene/water with 3.5% AcOH and 1.5% pyridine (1:3:4) (cf. [56]). After 60 transfers of upper and 5 of lower phase the product ($K \sim 6$) was isolated from tubes 43–60, the Boc derivative (31 mg) was dissolved in 80% aq. trifluoroacetic acid and, after 30 min, the solution was diluted with water, evaporated to small volume, rediluted with water, and evaporated to yield, on lyophilisation, 32 mg of material, essentially pure by tlc. Chromatography on a column (1.5 × 110 cm) of *Sephadex* G-15 in 0·2M AcOH gave a product which emerged in a symmetrical peak. It was recovered by evaporation and lyophilisation and dried for 10 h at 40° and 0.1 Torr. Yield 20 mg (46% from **12a**), $[\alpha]_D = -23.4°$ ($c = 0.58$, 1M AcOH), Rf 0.30 (A), 0.75 (C) (homogeneous in both systems), uterotonic activity 97.0 ± 14 IU/μmol. Amino-acid ratios: Asp 1.01 (1), Glu 1.09 (1), Pro 1.00 (1), Gly 1, ½Cys_2 1.90 (2), Ile 0.98 (1), Leu 1.02 (1), Phe (F) 1.01 (1).

$C_{43}H_{65}FN_{12}O_{11}S_2 \cdot C_2HF_3O_2 \cdot 5H_2O$	Calc.	C 44.55	H 6.32	N 13.85	H_2O 7.42%
(1213.3)	Found	,, 45.07	,, 6.37	,, 13.93	,, 7.24%

The presence of the two types of F atoms in the correct ratio was established by ^{19}F-NMR.

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45. Über die Struktur der makrocyclischen Spermidin-Alkaloide Oncinotin, Neooncinotin und Isooncinotin

151. Mitteilung über Alkaloide[1])

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(19.XII.73)

Summary. Three spermidine alkaloids – oncinotine (**1**), neooncinotine (**3**), and isooncinotine (**2**) – have been isolated from the stem bark of *Oncinotis nitida* BENTH. (*Scheme 1*); **1** and **3** are so far an unseparable mixture. However, by treatment of this mixture with K-*t*-butoxide, neooncinotine is completely converted into isooncinotine, and oncinotine, the main alkaloid, is obtained in pure form.

The structural assigment of these alkaloids is based on chemical and spectral evidence. Thus oncinotine (**1**) has been degraded *via* **24** (*Scheme 4*) and **32** to the putrescine derivative **35** and the piperidine derivative **34** (*Scheme 5*). Similarly neooncinotine (**3**) and isooncinotine (**2**), have given **34** along with the 1,3-diaminopropane derivative **36** (*Scheme 5*). The major decomposition pathways of **24**, **35** and **36** in the mass spectra are described in *Schemes 8, 6 and 7* respectively. The absolute configuration of **1**, **2** and **3** is derived by chiroptical correlations with (*R*)-(–)-N-methylconiine (**38**).

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[1]) 150. Mitt., vgl. [1].