eV) m/e 269 and 267 ($M^{18}O^+$, M^+), 239 and 237 (loss of 5'-CH_2O), 178 (adeninyl-$CH_2CH_2O^+$), 164 (adeninyl-CH_2O^+), 135 (adenine$^+$), 134 (adeninyl$^+$); ^{18}O content, 32.7 atom-%.

Adenosine-5'-^{18}O. Adenosine-5'-aldehyde[7] (50 mg, 0.19 mmol) slowly dissolved in a mixture of 0.6 ml of tetrahydrofuran and 0.250 g of $H^{18}OH$ (97%) when stirred for 16 hr at room temperature. $H^{18}OH$ was recovered as described above and the residue was suspended in 1 ml of tetrahydrofuran containing 0.050 g of $H^{18}OH$. The mixture was stirred for 1 hr with 0.10 g of $NaBH_4$; 0.5 ml of water was then added and stirring continued for 20 min when a clear solution had formed. Tetrahydrofuran was removed *in vacuo*, the aqueous solution neutralized with acetic acid and concentrated. The nucleoside was freed from salts by paper chromatography in butanol–water or by passage over a Dowex 1 × 2 (OH^-) column (1 × 30 cm) and elution with 60% methanol, according to the method of Dekker;[30] yield, 34 mg (68%); mass spectrum (11 eV) m/e 269 and 267 ($M^{18}O^+$, M^+), 237 (loss of 5'-$CH_2{}^{18}O$ and $CH_2{}^{16}O$), 164 (adeninyl-$CH_2CH_2O^+$), 135 (adenine$^+$), 134 (adeninyl$^+$); ^{18}O content 50.0 atom-%.

Adenosine-2'-^{18}O and -3'-^{18}O-5'-monophosphates were prepared by identical procedures.[25] A 3.23-g portion (10 mmol) of barium cyanoethyl phosphate was dissolved in water under addition of some Dowex 50 (H^+) and passed through a column of the same resin. The acidic eluate was freeze-dried and the colorless viscous product was further dried *in vacuo* over P_2O_5. The cyanoethyl phosphoric acid, dissolved in 4 ml of anhydrous dimethylformamide was added to a stirred suspension of 0.29 g (1.1 mmol) of adenosine-^{18}O and 8 ml of trimethyl orthoformate. After 2 hr at room temperature, a clear solution had resulted which was neutralized with pyridine and evaporated using an oil pump. The residue was dissolved in 10 ml of anhydrous pyridine and 2.1 g (10 mmol) of dicyclohexylcarbodiimide in 5 ml of pyridine was added. The reaction mixture was stirred overnight; dicyclohexyl urea was then removed by filtration and washed with pyridine–water (1:1). The filtrate was evaporated to dryness and treated with 100 ml of 9 N ammonia for 90 min at 60°, after which time the solution was filtered, extracted with ether and concentrated to half its volume. The aqueous solution was adjusted to pH 2.2 with concentrated formic acid (about 8 ml), kept for 4 hr at room temperature and lyophilized. The brown residue was redissolved in water and applied to a column (1 × 30 cm) of Dowex 1 × 2 (formate) and unreacted adenosine-^{18}O plus impurities were washed through with water. (The adenosine could best be recovered from the eluate by concentration and chromatography on two sheets of Whatman paper No. 3MM in butanol–water, yielding 0.30 mmol of nucleoside.) AMP-^{18}O was then eluted in a sharp peak with 0.1 M formic acid (pH 2.4) and the solution lyophilized; yield, 0.55 mmol (50%, determined optically) as 260 mg of a white solid with unknown water content; R_f 0.20 in solvent III.

(30) C. A. Dekker, *J. Amer. Chem. Soc.*, **87**, 4027 (1965).

Adenosine-2'- and -3'-^{18}O-5'-Triphosphates. By a published procedure,[26] 0.5 mmol of AMP-2'-^{18}O were converted in quantitative yield to the 5'-phosphoromorpholidate (R_f 0.58 in solvent III) which after drying over P_2O_5 was treated with 2.1 mmol of tri-*n*-butylammonium pyrophosphate in 8.1 ml of anhydrous dimethyl sulfoxide. After 2 days no more morpholidate could be detected by chromatography and the mixture was diluted with water and applied to a column (2 × 50 cm) of DEAE-cellulose (HCO_3^-). Elution with a triethylammonium bicarbonate gradient (0.05–0.45 M) gave 0.030 mmol of AMP-morpholidate, 0.065 mmol of AMP, 0.070 mmol of ADP, and 0.27 mmol (54%) of ATP-2'-^{18}O which was chromatographically pure (R_f 0.08 in solvent III). Similarly, 0.30 mmol of AMP-3'-^{18}O was converted to 0.19 mmol (63%) of ATP-3'-^{18}O. The triphosphates were rendered salt free by repeated evaporation *in vacuo* under addition of methanol and after drying over P_2O_5 were finally obtained as solid triethylammonium salts.

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The Synthesis of [1-Deamino,4-L-leucine]-oxytocin and [1-Deamino,4-L-isoleucine]-oxytocin and Some of Their Pharmacological Properties[1,2]

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Abstract: [1-Deamino,4-L-leucine]-oxytocin and [1-deamino,4-L-isoleucine]-oxytocin have been synthesized by the solid phase method, and some of their pharmacological properties have been determined. [1-Deamino,4-L-leucine]-oxytocin was also synthesized by the *p*-nitrophenyl ester stepwise procedure and compared to that synthesized by the solid phase method. The two preparations were found to be identical. The oxytocic and avian vasodepressor potencies of [1-deamino,4-L-leucine]-oxytocin and [1-deamino,4-L-isoleucine]-oxytocin are two- to threefold higher than those of [4-L-leucine]-oxytocin and [4-L-isoleucine]-oxytocin respectively. The two deamino analogs also exhibit milk-ejecting activities of approximately 150 units/mg. Neither analog possesses appreciable pressor or antidiuretic activity. A similar enhancement of oxytocic and avian vasodepressor activities had been observed when the free amino group of [4-L-valine]-oxytocin was replaced by hydrogen.

When the amino group of [4-valine]-oxytocin,[4] an analog of oxytocin (Figure 1), was replaced by hydrogen with the formation of [1-demino,4-valine]-oxytocin,[4] it was found that the deamino compound was strikingly more potent than [4-valine]-oxytocin, possessing more than three times the avian vasodepressor potency and more than twice the oxytocic potency of the parent compound. [1-Deamino,4-leucine]-oxytocin and [1-deamino,4-isoleucine]-oxytocin have now been prepared in order to compare their pharmacological properties with those of [4-leucine]-oxytocin and [4-

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(2) All optically active amino acid residues are of the L variety.
(3) To whom correspondence and reprint requests should be addressed.
(4) V. du Vigneaud, G. Flouret, and R. Walter, *J. Biol. Chem.*, **241**, 2093 (1966).

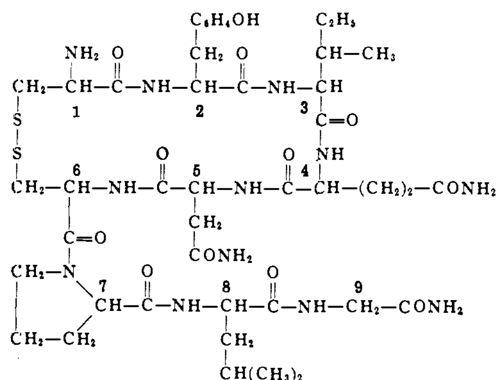

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues.

isoleucine]-oxytocin, which have already been reported.[5]

The two deamino analogs were synthesized by use of the solid phase method as described for the synthesis of deamino-oxytocin.[6] [1-Deamino,4-isoleucine]-oxytocin was also prepared by the stepwise *p*-nitrophenyl ester method as used in a synthesis of oxytocin.[7] Purification of the two analogs was accomplished by partition chromatography[8] and gel filtration[9] on Sephadex G-25.

The four-point assay design[10] was used for all bioassays against the USP posterior pituitary reference standard. Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by Munsick, Sawyer, and van Dyke.[11] Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of Holton,[12] as modified by Munsick,[13] with the use of magnesium-free van Dyke–Hastings solution as the bathing fluid. Milk-ejecting activity was measured on anesthetized rabbits by the method of Cross and Harris,[14] as modified by van Dyke, Adamsons, and Engel,[15] and Chan.[16] Pressor assays were carried out on anesthetized male rats as described in the United States Pharmacopeia.[17] Assays for antidiuretic activity were performed on anesthetized male rats according to the method of Jeffers, Livezey, and Austin,[18] as modified by Sawyer.[19]

The pharmacological activities presented in Table I show that [1-deamino,4-leucine]-oxytocin and [1-deamino,4-isoleucine]-oxytocin are considerably more potent than [4-leucine]-oxytocin and [4-isoleucine]-oxytocin, respectively, in avian vasodepressor and oxytocic activities, as was also observed in the case of the comparable valine analogs of oxytocin.

In the milk-ejecting assay, [1-deamino,4-leucine]-oxytocin was found to be far more active than [4-leucine]-oxytocin, whereas in the case of [1-deamino,4-isoleucine]-oxytocin and [4-isoleucine]-oxytocin both analogs possessed approximately the same potency. For these four analogs the potency with respect to pressor and antidiuretic activity is extremely low or nil. It is of particular interest that [4-leucine]-oxytocin exhibits a diuretic effect. Furthermore, it has been shown that [4-leucine]-oxytocin is not only a potent diuretic agent but possesses a natriuretic effect and antagonizes the antidiuretic activity of the antidiuretic hormone arginine-vasopressin.[20]

Table I. Pharmacological Potencies of [4-Valine]-oxytocin, [4-Leucine]-oxytocin, [4-Isoleucine]-oxytocin, and Their Deamino Analogs[a]

Compound	Vasodepressor (fowl)	Oxytocic (rat)	Milk-ejecting (rabbit)	Antidiuretic (rat)	Pressor (rat)
[4-Valine]-oxytocin[b]	230 ± 14	139 ± 5	~419	~0.5	<0.005
[1-Deamino,4-valine]-oxytocin[b]	770 ± 25	322 ± 16	338 ± 2	4.5 ± 0.4	<0.005
[4-Leucine]-oxytocin[c]	44 ± 1	13 ± 1	66 ± 3	Diuretic	Weak depressor
[1-Deamino,4-leucine]-oxytocin	141 ± 9	37 ± 1	143 ± 4	~0.015	<0.05
[4-Isoleucine]-oxytocin[c]	81 ± 2	~37	~184	~0.02	<0.05
[1-Deamino,4-isoleucine]-oxytocin	307 ± 24	67 ± 1	~156	<0.05	~0.06

[a] Expressed in units per milligram as mean potencies ± standard error. [b] W. Y. Chan and V. du Vigneaud, unpublished data. Approximate values were reported in ref 4. [c] See ref 5.

Experimental Section

S-Benzyl-β-mercaptopropionyl-O-benzyltyrosylisoleucylleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide. Nitrated chloromethylcopolystyrene–2% divinylbenzene resin was prepared and esterified with Boc-glycine by the procedures reported earlier.[6] Amino acid analysis of an acid hydrolysate (dioxane, 12 *N* HCl, 1:1) showed the product to contain 0.38 mmol of glycine/g of esterified resin. The Boc-glycyl nitrated resin (2 g) was placed in the Merrifield reaction vessel. For the introduction of each new amino acid residue, the cycle of deprotection, neutralization, and coupling described in the earlier communication[6] was followed.

For the washing steps 10-ml portions of the appropriate solvent were used. In the deprotection step 1 *N* HCl in glacial acetic acid (10 ml) was used, and 1.4 ml of triethylamine in 10 ml of dimethylformamide was used for the neutralization of the hydrochloride. In the coupling steps 2.6 mmol of the appropriate Boc-amino acid in 7 ml of methylene chloride and 2.6 mmol of N,N′-dicyclohexylcarbodiimide in 3 ml of methylene chloride were used. The coupling reaction involving Boc-asparagine was carried out *via* its *p*-nitrophenyl ester (2.6 mmol in 6 ml of distilled dimethylformamide) with a reaction time of 5 hr.

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(19) W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).
(20) W. Y. Chan, V. J. Hruby, G. Flouret, and V. du Vigneaud, *Science*, **161**, 280 (1968).

Following the incorporation of the S-benzyl-β-mercaptopropionic acid residue, the protected polypeptide-nitrated resin compound was further washed with 10-ml portions of glacial acetic acid (three times), absolute ethanol (three times), and methylene chloride (three times). The product was dried *in vacuo* over KOH pellets; yield, 2.6 g.

Dry ammonia was bubbled into a stirred suspension of 2.56 g of the protected polypeptide-nitrated resin in 100 ml of anhydrous methanol at 0° for 2 hr (until the solution was saturated with ammonia). The reaction mixture was stirred overnight at 0–6°. The methanol and ammonia were removed under aspirator vacuum. Dimethylformamide (125 ml) was added to the dry residue and the suspension was stirred vigorously for 2 hr. The resin was filtered off and washed twice with dimethylformamide.

The solvent was removed from the combined filtrate and washings on a rotary evaporator at below 40°. The residue was triturated with ethyl acetate, and the cream-colored solid was filtered off and washed with ethyl acetate. The product was ground to a powder in about 25 ml of methanol, filtered off, washed with methanol, and dried *in vacuo* over KOH pellets; yield, 305 mg, mp 236–239° (dec).

A sample was prepared for analysis by precipitation from a dimethylformamide–water mixture. The compound was filtered off, washed with methanol, and dried *in vacuo* over KOH pellets; mp 241–243° (dec), $[\alpha]^{18}D$ −43.3° (*c* 1.1, dimethylformamide).

Anal. Calcd for $C_{65}H_{88}N_{10}O_{11}S_2$: C, 62.5; H, 7.10; N, 11.2. Found: C, 62.0; H, 7.11; N, 11.1.

[1-Deamino,4-leucine]-oxytocin. S-Benzyl-β-mercaptopropionyl-O-benzyltyrosylisoleucylleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide (237 mg) was dissolved in 150 ml of stirred boiling liquid ammonia (distilled from sodium in an all-glass apparatus). A fresh sodium stick was momentarily introduced intermittently until the blue color persisted for 30 sec. The ammonia was evaporated off at the water aspirator and the last 50 ml was removed by lyophilization. The lyophilized residue was dissolved in 500 ml of 0.03% trifluoroacetic acid. The pH of the solution was adjusted to 8.0 with ammonium hydroxide, and an excess of 0.1 *N* potassium ferricyanide (3.8 ml) was added to the stirred solution. After 15 min AG 3-X4 resin (chloride form) was added, and stirring was continued for 15 min to remove ferrocyanide and excess ferricyanide ions. The resin was removed by filtration and the solution was lyophilized.

The residue was dissolved in 25 ml of upper phase of the solvent system 1-butanol–benzene–3.5% aqueous acetic acid (containing 1.5% pyridine) (1:3:4) and applied to a Sephadex G-25 (100–200 mesh) column (2.80 × 60 cm) that had been equilibrated with the lower and upper phases. The column was eluted with the upper phase and 90 fractions of 9.5 ml each were collected. The chromatogram obtained by plotting the Folin-Lowry color values[21] of the fractions showed a single peak with a maximum at fraction 18 (R_f 0.52). The fractions corresponding to this peak were pooled, twice the volume of water was added, and the resulting mixture was concentrated under reduced pressure and lyophilized.

The lyophilized powder (64 mg) was dissolved in 15 ml of 0.2 *N* acetic acid and subjected to gel filtration[9] on a Sephadex G-25 (200–270 mesh) column (2.82 × 68 cm) that had been equilibrated with 0.2 *N* acetic acid. The column was eluted with 0.2 *N* acetic acid, and 100 fractions of 5.3 ml each were collected. A plot of the Folin-Lowry color values of the various fractions showed a single symmetrical peak with a maximum at fraction 64. The fractions corresponding to this peak were pooled and lyophilized to give a white powder; yield, 44 mg, $[\alpha]^{24}D$ −86.2° (*c* 0.5, 1 *N* acetic acid).

Anal. Calcd for $C_{44}H_{68}N_{10}O_{11}S_2$: C, 54.1; H, 7.01; N, 14.3. Found: C, 54.1; H, 7.08; N, 14.2.

A sample was hydrolyzed for 42 hr in 6 *N* HCl at 110° and analyzed on a Beckman-Spinco amino acid analyzer, according to the method of Spackman, Stein, and Moore.[22] The following molar ratios were obtained, glycine being taken as 1.0: aspartic acid, 1.0; proline, 1.0; glycine, 1.0; isoleucine, 1.1; leucine, 2.0; tyrosine, 1.0; and ammonia, 2.0. In addition, cystine, 0.25 and the mixed disulfide[23] of cysteine and β-mercaptopropionic acid, 0.6, were present. These two sulfur-containing compounds thus account for the half-cystine residue in the analog. A 42-hr hydrolysis time was necessary for complete hydrolysis of the isoleucyl–leucine peptide bond.

S-Benzyl-β-mercaptopropionyltyrosylisoleucylisoleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide. A stirred solution of 2.41 g of N-benzyloxycarbonyl-O-benzyltyrosylisoleucylisoleucylasparaginyl-S-benzyl-cysteinylprolylleucylglycinamide[5] in 40 ml of anhydrous acetic acid was treated with 40 ml of 5.4 *N* HBr in anhydrous acetic acid for 1 hr at room temperature. Ether (400 ml) was then added and the precipitate was filtered off, washed with three 150-ml portions of ether, and dried *in vacuo* over P_2O_5; yield, 2.72 g of the free octapeptide salt. A solution of 1.36 g of the salt in 20 ml of dimethylformamide was neutralized to pH 6.0 with triethylamine, and 0.32 g of *p*-nitrophenyl S-benzyl-β-mercaptopropionate[23] was added. The mixture was stirred for 24 hr at room temperature and the resulting slurry was triturated with 200 ml of cold ethyl acetate. The product was filtered off and washed with 30-ml portions of ethyl acetate (two times), 25-ml portions of ethanol (two times), 25-ml portions of 50% ethanol (two times), and 30-ml portions of ethyl acetate (two times), and then dried *in vacuo;* yield, 1.08 g, mp 258–259° (dec), $[\alpha]^{23}D$ −45.0° (*c* 0.5, dimethylformamide).

An analytical sample was prepared by precipitation of the product from a dimethylformamide solution with water.

Anal. Calcd for $C_{58}H_{82}N_{10}O_{11}S_2$: C, 60.1; H, 7.13; N, 12.1. Found: C, 59.8; H, 7.31; N, 11.8.

[1-Deamino,4-isoleucine]-oxytocin. S-Benzyl-β-mercaptopropionyltyrosylisoleucylisoleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide (232 mg) in 175 ml of freshly distilled ammonia was treated at the boiling point with sodium until a blue color persisted for 30 sec. During this time complete solution of the polypeptide resulted. The ammonia was removed by evaporation and lyophilization. The colorless salt was dissolved in 600 ml of deaerated water containing 0.50 ml of trifluoroacetic acid. The pH was adjusted to 8.2 with dilute ammonia and the solution was treated with 35 ml of 0.01 *N* potassium ferricyanide. After 30 min the pH was adjusted to 6.0 with dilute trifluoroacetic acid and the resulting cloudy solution was treated with AG 3-X4 resin (chloride form). After the mixture was stirred for 20 min, the resin was filtered off and the clear filtrate was lyophilized.

The colorless residue was dissolved in 5 ml of 1-butanol and 2 ml of the lower phase of the solvent system 1-butanol–benzene–3.5% aqueous acetic acid (containing 1.5% pyridine) (1:3:4). Benzene (15 ml) was added, and the solution was placed on a Sephadex G-25 (100–200 mesh) column (2.8 × 64 cm) that had been equilibrated with the lower and upper phases.[8] Fifty 10-ml fractions were collected and the fractions corresponding to the major peak (R_f 0.48) as determined by Folin-Lowry color values were pooled, water (300 ml) was added, and the mixture was concentrated to about 50 ml and lyophilized. The resulting white powder (31 mg) was dissolved in 8 ml of 0.2 *N* acetic acid and placed on a Sephadex G-25 (200–270 mesh) column (2.8 × 65 cm) for gel filtration. One hundred 6.8-ml fractions were collected, and the fractions corresponding to the major peak with a maximum at fraction 49, as determined by Folin-Lowry color values, were pooled and lyophilized to give 27.8 mg of [1-deamino,4-isoleucine]-oxytocin as a white powder, $[\alpha]^{24}D$ −88.2° (*c* 0.5, 1 *N* acetic acid).

Anal. Calcd for $C_{44}H_{68}N_{10}O_{11}S_2$: C, 54.1; H, 7.01; N, 14.3. Found: C, 54.4; H, 7.23; N, 14.2.

A sample was hydrolyzed for 65 hr in 6 *N* HCl at 110° and analyzed on a Beckman-Spinco amino acid analyzer. The following molar ratios were obtained, glycine being taken as 1.0: aspartic acid, 1.0; proline, 1.0; glycine, 1.0; cystine, 0.23; mixed disulfide of cysteine and β-mercaptopropionic acid, 0.65; isoleucine, 2.0; leucine, 1.0; tyrosine, 1.0; and ammonia, 2.0. Prolonged hydrolysis was necessary because of the difficulty in the hydrolysis of an isoleucyl–isoleucine peptide bond.[24,25]

Solid Phase Synthesis of S-Benzyl-β-mercaptopropionyl-O-benzyltyrosylisoleucylisoleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide and Its Conversion to [1-Deamino,4-isoleucine]-oxytocin. S-Benzyl-β-mercaptopropionyl-O-benzyltyrosylisoleucylisoleucylasparaginyl-S-benzylcysteinylprolylleucylglycyl nitrated resin was prepared by treatment of 2 g of Boc-glycyl nitrated resin (0.42 mmol of glycine/g of esterified resin) in an 8-cycle procedure as already described for the synthesis of S-benzyl-β-mercaptopropionyl-

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O-benzyltyrosylisoleucylleucylasparaginyl-S-benzylcysteinylprolylleucylglycyl-nitrated resin.

Dry ammonia was bubbled into a stirred suspension of the protected polypeptide-nitrated resin in 100 ml of anhydrous methanol at 0° until the solution was saturated with ammonia. The reaction mixture was then stirred overnight at 2°, and the solvent and ammonia were carefully removed by evaporation *in vacuo*.

The resulting dry powder was stirred with 150 ml of dimethylformamide at 110° for 20 min. The resin was then filtered off and washed with two 10-ml portions of dimethylformamide. The combined filtrate and washings were evaporated to dryness *in vacuo*, and the resulting oil was triturated with water. The product was filtered off and washed with methanol to give a pale tan powder (0.55 g). Precipitation from a dimethylformamide solution with ethanol gave 0.49 g of the protected polypeptide amide as a white powder, mp 256–258° (dec), $[\alpha]^{24}D$ −42.7° (*c* 0.5, dimethylformamide).

Anal. Calcd for $C_{65}H_{88}N_{10}O_{11}S_2$: C, 62.5; H, 7.10; N, 11.2. Found: C, 62.1; H, 7.09; N, 11.0.

[1-Deamino,4-isoleucine]-oxytocin was prepared from the preceding compound (250 mg) by the same procedures used for the preparation of this analog from the protected polypeptide that had been synthesized by the *p*-nitrophenyl ester method. The analog was obtained as a white powder; yield, 50.1 mg, $[\alpha]^{24}D$ −88.2° (*c* 0.5, 1 *N* acetic acid).

Anal. Calcd for $C_{44}H_{68}N_{10}O_{11}S_2$: C, 54.1; H, 7.01; N, 14.3. Found: C, 54.0; H, 7.02; N, 13.9.

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Carbon-13 Magnetic Resonance Studies of Amino Acids and Peptides. II

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Abstract: Carbon-13 (C-13) nuclear magnetic resonance studies of C-13 enriched (15%) amino acids are reported. The amino acids were isolated from C-13 enriched algae grown on $^{13}CO_2$. The spectrometer used operates either in the continuous wave or pulse mode. In the latter mode, the Fourier transform of the C-13 resonances is obtained. The C-13 nuclei were generally noise decoupled from the protons. The observed chemical shifts are compared with those predicted on the basis of empirical rules. The carbon chemical shifts in parts per million are more than an order of magnitude larger than the corresponding proton shifts of the amino acids.

A number of proton magnetic resonance (pmr) studies of enzyme conformation and enzyme-inhibitor interactions have recently been reported.[2–7] Interest in the pmr technique has been stimulated by new instrumental developments, particularly the availability of commercial high-resolution superconducting magnets operating at *ca.* 52,000 G. Proton resonance spectra of proteins are difficult to interpret. The number of protons per enzyme is very large, while the intrinsic line widths are generally broad, exceeding 10 Hz, and the chemical shift spread is relatively small. In an effort to circumvent these difficulties, Jardetzky, *et al.*,[7] and Katz, *et al.*,[8] have prepared deuterated enzymes which contain one or more amino acids in the normal proton form. This approach gives significantly simplified pmr spectra and permits studies not otherwise possible.

It would appear that C-13 labeling of proteins offers an alternate or complementary approach. One expects large chemical shifts and narrow line widths relative to the pmr case. C-13 labeling is a positive approach as contrasted with deuterium labeling[7,8] in the sense that its own resonance is observed. Also relatively small quantities of isotopic material are required. The range of systems which can be potentially C-13 labeled is large, and include such diverse systems as tissue cultures and bacteria.[9]

In this paper we present magnetic resonance data of most of the naturally occurring amino acids using direct C-13 detection at 15 MHz. This work is an extension of an earlier indirect, *i.e.*, INDOR study of the C-13 resonances.[10] Measurements were done mainly in D_2O under high-resolution conditions using noise[11] or coherent decoupling of the protons from the carbons. No carbon–proton coupling constants are reported. Both continuous and pulse techniques were employed. The results are contrasted with the pmr measurements at *ca.* 52,000 G.[12]

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