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## Synthesis and Some Pharmacological Properties of 8-ε-Hydroxynorleucine-vasopressin†

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In both lysine-vasopressin and arginine-vasopressin, the eighth position in the chain is occupied by a basic acid. *A priori*, it seems obvious that the cation-forming group in the side chain of these basic amino acids is involved either in binding to the (unknown) receptor or in triggering hormonal action. Earlier work has shown that replacement of the basic guanido group of arginine-vasopressin by the neutral ureido group led to an analog, 8-L-citrulline-vasopressin[2] that was still active although less potent than the natural hormone. Similarly, some light was shed on the role of the side-chain amino group in the pharmacological properties of lysine-vasopressin in the studies involving 8-$N^{\epsilon}$-formyllysine-vasopressin.[3] The moderate hormonal activities reported for this analog did not permit firm conclusions. While the formylamino group cannot be readily protonated in this compound, the decrease in potency could be due to reasons other than lack of ionic bond formation; *e.g.*, the protecting group might prevent the necessary molecular fit between hormone and receptor. There is a possibility that the amino group in question participates in the hormone-receptor interaction by hydrogen bonding rather than by an ionic bond. In the work reported here, this question was examined by replacing L-lysine in the hormone with an ε-hydroxynorleucine residue.

Recently, in a similar study Hope and Walti[4] replaced the single free amino group of the N-terminal cysteine residue of oxytocin with a hydroxyl group and obtained an oxytocin analog of remarkable potency. Their considerations also involve hydrogen bonding with the receptor.

**Synthesis.** The new analog, 8-ε-hydroxynorleucine-vasopressin (I), was synthesized by the stepwise approach[5] first applied in the synthesis of oxytocin[6] and lysine-vasopressin.[7] The ε-hydroxynorleucine (II) was prepared and resolved by the methods of Gaudry[8] and Berlinguet and Gaudry[9] with the modifications described in the Experimental Section.

An attempt toward a synthesis using an unprotected hydroxyl group of the ε-hydroxynorleucine residue failed. On exposure to the relatively mild acidolytic conditions required for the removal of the *tert*-butyloxycarbonyl groups, the partially protected dipeptide derivative, *N*-benzyloxycarbonylhydroxynorleucylglycine ethyl ester, was cleaved with the liberation of glycine ethyl ester. This not quite unexpected[10] intramolecular N → O acyl migration rendered the protection of the ε-hydroxyl group mandatory. With ε-acetoxynorleucine, the synthesis proceeded to the completely protected nonapeptide without difficulties. The *O*-acetyl group was removed by hydrazinolysis, and the N-terminal benzyloxycarbonyl group and the two *S*-benzyl groups were removed by reduction with sodium in liquid ammonia. The cyclic disulfide was formed by air oxidation. After desalting on a Sephadex G25 column, the hormone analog was purified by countercurrent distribution through 1000 transfers. Two peaks were obtained; the one with higher distribution coefficient corresponded to the desired hormone analog I. The slower migrating material was identified as the dimer of I by its lower solubility and diffusion rate, and particularly by its high specific rotation.[11,12] When the hydrazinolysis was omitted, I was also obtained as the major product but then, in addition to the dimer peak, a smaller, faster moving peak was also found on analysis of the countercurrent distribution fractions. The material isolated from this peak had the properties to be expected of 8-ε-acetoxynorleucine-vasopressin (III).

**Biological Activities.** Vasopressor activity was measured by the Dekanski[13] method in rats under urethane anesthesia pretreated with phenoxybenzene. Eight groups of four rats were used. No significant deviation of slope compared to the standard was observed. 8-ε-Hydroxynorleucine-vasopressin had vasopressor activity equivalent to 31 ± 1 U/mg. Antidiuretic activity estimated by intravenous injection into hydrated rats under ethanol anesthesia[14] was about 75 U/mg. The USP posterior pituitary reference standard was used in both assays.

**Conclusion.** The pressor and antidiuretic effects exhibited by the new analog demonstrate that the amino group in the side chain of lysine-vasopressin is not essential for biological activity. The fact that the analog is less potent than the parent hormone indicates that a hydrogen bond either is not involved or is less effective than an ionic bond between hormone and receptor.

### Experimental Section

**General Comments.** Melting points were taken in capillary tubes and are uncorrected. Where analyses are indicated by only the symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values. For tlc, precoated plates (E. Merck AG, Darmstadt) were used and peptides were detected by the use of uv, ninhydrin, chlorination,[15] and charring[16] techniques. The following solvent systems were used: A, *n*-BuOH–AcOH–$H_2O$ (4:1:1); B, EtOAc–pyridine–AcOH–$H_2O$ (60:20:6:11); C, $CHCl_3$–MeOH (9:1). Circular paper chromatograms were run on Whatman No. 1 paper in the upper phase of *n*-BuOH–AcOH–$H_2O$ (4:1:5) and the peptides were detected with ninhydrin. Proton nmr spectra were recorded in a Varian A-60 analytical spectrometer. Chemical shifts are downfield from the standard $Me_4Si$.

For quantitative amino acid analysis, samples were hydrolyzed with 6 *N* HCl in evacuated, sealed ampoules at 110° for 16 hr and analyzed by the Spackman–Stein–Moore method[17] on a Beckman Spinco 120C amino acid analyzer. II is unstable under hydrolysis conditions. A variable mixture consisting of 10–30% amino acid and the rest another compound, presumably the lactone, is obtained. (Lactone is known to form from the acid on treatment with HCl.[9]) The lactone appeared as a distinct peak just before tyrosine but well separated from it. A constant for this product was calculated after subjecting known amounts of free acid to hydrolysis conditions in which the time of hydrolysis was varied from 16 to 48 hr before amino acid analysis. The constant for the acid itself was determined by analyzing a sample of known weight without prior hydrolysis. In addition, the ninhydrin product of II coincided with that of glycine. This was demonstrated in known mixtures of standard and II. A composite constant determined for mixtures of glycine and I was used in all subsequent determinations.

The properties of the compounds whose preparation is described below are summarized in Tables I and II.

**ε-Hydroxy-DL-norleucine (IV).** The method of Gaudry[8] was followed through the preparation of 5-ε-hydroxybutylhydantoin. Subsequent hydrolysis was carried out by refluxing for 1–2 days with either concentrated NaOH or concentrated $Ba(OH)_2$. When

†Unless otherwise indicated, all optically active amino acid residues have the L configuration. The symbols for the amino acid residues follow the tentative rules of the IUPAC-IUB Commission on Biomedical Nomenclature.[1] The symbol, Hyn, is used to stand for ε-hydroxynorleucine.

**Table I.** Properties of Compounds[a]

| Compd[e] | Mp, °C Crude | Mp, °C Recrystd | $R_f$ Paper[c] Rd | $R_f$ Paper[c] Dec | $R_f$ Tlc[d] A | $R_f$ Tlc[d] B | $R_f$ Tlc[d] C | $[\alpha]^{25}D$, deg c | $[\alpha]^{25}D$, deg Solvent | $[\alpha]^{25}D$, deg Value | Formula | Analyses[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IV | 230–231[f] | | | | | | | | | | | |
| | 236–239[g] | 258[h] | 0.38 | | | | | | | | | |
| II | 237–238 | | 0.38 | | | | | 2 | 6 *N* HCl | +23.5[i] | | |
| V | | 233–237[j] | 0.58 | | | | | 2.2 | 6 *N* HCl | +18.9 | $C_8H_{15}NO_4$ | C, H, N |
| VIII | | 136–137[k] | | | | 0.66 | 0.23 | 2, 4.7 | AcOH | −2, −2 | $C_{18}H_{25}N_3O_6$ | C, H, N |
| IX | 144–146 | 147–149[l] | | | | 0.53 | 0.28 | 2 | AcOH | −64 | $C_{23}H_{32}N_4O_7$ | C, H, N |
| XI | 79–83 | | | | 0.60 | | 0.34 | 2 | Abs EtOH | −44 | | |
| X | 176–179 | 182–183[m] | | | 0.50 | | | 2 | AcOH | −61 | $C_{37}H_{49}N_7O_{10}S$ | C, H, N, S |
| XII | 196–199 | 197–199[m] | | | 0.43 | | | 2 | AcOH | −64 | $C_{42}H_{57}N_9O_{12}S$ | C, H, N, S |
| | | | | | | | | | | | $C_{42}H_{57}N_9O_{12}S \cdot 0.5H_2O$ | C, H, N, S |
| XIII | 206–208 | 207–208[m] | | | 0.46 | | | 2 | AcOH | −54 | $C_{51}H_{66}N_{10}O_{13}S$ | C, H, N, S |
| | | | | | | | | | | | $C_{51}H_{66}N_{10}O_{13}S \cdot H_2O$ | C, H, N, S |
| XIV | 237–239 | 243–244[m] | | | 0.50 | | | 2 | AcOH | −48 | $C_{67}H_{81}N_{11}O_{15}S$ | C, H, N, S |
| XV | 227–229 | 227–228[m] | | | 0.33 | 0.40 | | 2 | AcOH | −50 | $C_{70}H_{86}N_{12}O_{16}S_2$ | C, H, N, S |
| XVI | 229–231 | | | | 0.29 | 0.38 | | 0.8 | AcOH | −51 | $C_{68}H_{84}N_{12}O_{15}S_2 \cdot 2H_2O$[n] | C, H, N, S |
| Fraction 1[o] | | | | 0[p] | 0 | 0.07 | | 0.7 | 1 *N* AcOH | −56 | | |
| | | | | 0.38[q] | | | | | | | | |
| Fraction 2,[o] I | | | | 0.13[p] | 0.18 | 0.38 | | 1.1 | 1 *N* AcOH | −27 | | |
| | | | | 0.61[q] | | | | | | | | |

[a]Details for determining the properties were given in the Experimental Section. [b]Where two calculated structures are given, the analysis seemed to fit both almost equally well. [c]Rd indicates round papers were used. Dec indicates that a descending paper chromatogram was run. All the samples described were homogeneous under the conditions tested. [d]The letters correspond to the systems described at the beginning of the Experimental Section. [e]The compounds are listed in the order in which they appear in the Experimental Section. [f]From NaOH hydrolysis. [g]From $Ba(OH)_2$ hydrolysis. [h]A sample was recrystallized from $H_2O$–MeOH (50:50). The melting point reported by Berlinguet and Gaudry[9] was 248°. [i]Berlinguet and Gaudry[9] report +23.7°. [j]A sample was purified by dissolving in AcOH containing 1 g of $HClO_4$, centrifuging, adding 1 g of hexylamine, and pouring into $Et_2O$. It was isolated by centrifuging and then dried under vacuum at 85° for 3 hr. [k]After countercurrent distribution and drying overnight *in vacuo* at 40°. [l]A sample was recrystallized from EtOAc–hexane. [m]A sample was purified by dissolving in AcOH, centrifuging, and reprecipitating in $Et_2O$. It was dried *in vacuo* over $P_2O_5$ at 50°. [n]Loss of weight on drying at 110° was 2.7%. [o]Fractions 1 and 2 were obtained from the countercurrent distribution carried out after the first preparation of I. [p]$R_f$ value obtained using the system *n*-BuOH–AcOH–$H_2O$ (4:1:5). [q]$R_f$ value obtained using the system *n*-BuOH–Py–AcOH–$H_2O$ (30:20:6:24).

**Table II.** Amino Acid Analyses[a]

| Compd | Asp | Glu | Pro | Gly + Hyn | Cys | Phe | Tyr | Hyn-lactone[b] | Cys-Bzl |
|---|---|---|---|---|---|---|---|---|---|
| VIII | | | | 1.2 | | | | 0.80 | |
| IX | | | 0.93 | 1.3 | | | | 0.77 | |
| X | 1.0 | | 1.0 | 1.4 | | | | 0.80 | 0.91 |
| XII | 1.0 | 1.1 | 0.99 | 1.2 | | | | 0.86 | 0.98 |
| XIII | 1.0 | 1.1 | 0.91 | 1.2 | | 1.2 | | 0.75 | 0.99 |
| XIV | 1.2 | 0.94 | 1.2 | 1.3 | | 0.90 | 1.0 | 0.75 | 0.80 |
| XV | 1.1 | 1.1 | 0.92 | 1.3 | | 1.0 | 1.1 | 0.85 | 1.8 |
| XVI | 1.1 | 0.90 | 1.0 | 1.2 | | 1.0 | 1.1 | 0.95 | 1.9 |
| Fraction 1[c] | 1.0 | 1.1 | 1.0 | 1.3 | 2.0 | 1.1 | 0.85 | 0.75 | |
| Fraction 2,[c] I | 1.0 | 1.1 | 0.95 | 1.3 | 2.0 | 1.1 | 0.90 | 0.80 | |
| Fraction 3[d] | 1.0 | 1.0 | 0.97 | 1.3 | 2.0 | 0.98 | 0.83 | 0.72 | |

[a]Details concerning the amino acid analyses are given at the beginning of the Experimental Section. [b]Hyn-lactone is used to designate the lactone formed from Hyn on acid hydrolysis. [c]Fractions 1 and 2 were obtained from the countercurrent distribution carried out after preparation 1 to I. [d]Fraction 3 was isolated from the countercurrent distribution carried out after preparation 2 of I.

NaOH was used, the product was isolated in 50% yield; a yield of 94% could be obtained by using $Ba(OH)_2$. In a typical experiment, the hydantoin (43 g, 0.25 mol) was heated under reflux in a stainless steel vessel with $Ba(OH)_2 \cdot 8H_2O$ (152 g, 0.48 mol) in $H_2O$ (750 ml) for 48 hr. The solution was cooled, filtered, treated with $(NH_4)_2CO_3$ (30 g), and evaporated to dryness.

**ε-Hydroxynorleucine (II)** was prepared by treatment of the *N*-chloroacetyl derivative of IV (an oil) with acylase according to the procedure of Berlinguet and Gaudry.[9] The amino acid was secured in poor yield (*ca.* 10%).

**ε-Acetoxynorleucine (V)** was prepared from II according to the method described for *O*-acetyl-DL-serine.[18] $HClO_4$ (3.2 g, 70%) and $Ac_2O$ (99%, 1.8 g) were mixed and diluted to 35 ml with AcOH previously treated with $Ac_2O$ (1 ml). The hydroxyamino acid II (2.06 g, 14 mmol) was added and the mixture was stirred until solution occurred and then cooled in an ice bath. More $Ac_2O$ (99%, 7.5 g) was added. After 2 hr, the mixture was allowed to come to room temperature and $H_2O$ (1 ml) was mixed in. Stirring was continued for 1.5 hr and then hexylamine (2.9 g) was dropped in with cooling. On dilution with anhydrous $Et_2O$ (300 ml), a precipitate formed. The solid (2.46 g, 93%) was filtered off and washed with $Et_2O$. In the nmr spectrum ($D_2O$), a 3-proton singlet at 2.02 ppm corresponds to the methyl protons of the acetyl group.

**Z-Gly-$NH_2$ (VI).** A gentle stream of $NH_3$ was passed over a stirred solution of Z-Gly-ONP (9.9 g, 30 mmol) in THF (150 ml) for 2 hr. The solution was poured into anhydrous $Et_2O$ (500 ml); the precipitate was filtered off and washed with $Et_2O$. The amide (5.64 g, 91%) melts at 137–138° (lit.[19] 137–138°).

**Gly-$NH_2$·HBr (VII)** was prepared as described earlier.[19]

**Z-Hyn(Ac)-Gly-$NH_2$ (VIII).** Compound V was converted to the Z derivative by treating a solution of the acetoxyamino acid in aqueous $NaHCO_3$ with benzyloxycarbonyl chloride, according to the procedure described by Greenstein and Winitz[20] for Z-Thr. An oil was obtained in almost quantitative yield; it was characterized by its nmr spectrum ($CDCl_3$) that showed, in addition to the peaks exhibited by V, a 2-proton singlet at 5.10 ppm and a 5-proton singlet at 7.36 ppm. This material (3.86 g, 12 mmol) was dissolved in $CH_2Cl_2$ (20 ml) and added to a solution of VII (from 3.12 g of VI, 15 mmol) in $CH_2Cl_2$ (20 ml). An additional amount (10 ml) of $CH_2Cl_2$ was used for rinsing. The mixture was stirred and cooled in an ice–water bath, and dicyclohexylcarbodiimide (2.46 g, 12 mmol) was added. After 30 min at 0°, the mixture was stirred at room temperature for 2.5 hr. The solids were removed by filtration, washed with $Et_2O$, EtOAc, and $H_2O$, and purified by countercurrent distribution. The filtrate together with the $Et_2O$ and EtOAc washings was evaporated to dryness, the residue was taken up in EtOAc and filtered, and the solution was washed with dilute HCl, $H_2O$, and saturated $NaHCO_3$ and $H_2O$. After drying over $MgSO_4$, the solvent was removed and the residue was purified also by countercurret distribution. The solvent system $CHCl_3$–toluene–$CH_3OH$–$H_2O$ (5:5:8:2) was used in a 60-tube Craig apparatus with 10-ml phases. After 60 transfers, the protected dipeptide amide was isolated from a band corresponding to $k = 3.2$. The distribution curve was in close agreement with the curve calculated for this value. The combined yield of purified VIII was 2.1 g (47%). The nmr spectrum ($CD_3COOD$) is consistent with the expected structure.

**Z-Pro-Hyn(Ac)-Gly-$NH_2$ (IX).** To a solution of VIII (2.1 g, 5.55 mmol) in AcOH (25 ml), 4 *N* HBr in AcOH (25 ml) was added. After 1 hr at room temperature, the hydrobromide was precipitated with $Et_2O$ (450 ml), filtered, washed with $Et_2O$, and dried briefly *in vacuo* before dissolution in DMF (13 ml). First $Et_3N$ (2 ml) was added and then Z-Pro-ONP[6] (2.64 g, 7.15 mmol). The reaction was allowed to proceed at room temperature. Small amounts of $Et_3N$ were added to keep the solution basic. After 16 hr, the solvent was removed *in vacuo*, the residue was dissolved in $CHCl_3$ (40 ml), and the solution was washed with $H_2O$ (3 × 10 ml). The organic layer was dried over $MgSO_4$ and evaporated to dryness. To a solution of the residue in EtOAc, hexane was added to turbidity. On cooling, the product separated: 1.85 g (70%). The expected nmr spectrum was observed ($CDCl_3$).

**Z-Asn-Cys(Bzl)-Pro-Hyn(Ac)-Gly-$NH_2$ (X).** The protected tripeptide amide IX (0.48 g, 1.0 mmol) was treated with HBr in AcOH as described above. The resulting hydrobromide was treated with diisopropylethylamine[21] and acylated with Z-Cys(Bzl)-ONP.[6] The product Z-Cys(Bzl)-Pro-Hyn(Ac)-Gly-$NH_2$ (XI) (0.50 g, 74%) was obtained in crystalline form on prolonged standing under $Et_2O$. It gave an nmr spectrum ($CDCl_3$) that was consistent with the expected structure. This material was used without purification for the preparation of the protected pentapeptide amide X. Removal of the Z group (from 1.0 g, 1.5 mmol) with HBr in AcOH yielded the hydrobromide that was dissolved in DMF (5.0 ml) and treated with $Et_3N$ (0.8 ml) until basic and then with Z-Asn-ONP[6] (1.16 g, 3.0 mmol). The next day the solvent was removed *in vacuo;* the solid residue was disintegrated under THF, filtered and washed with $H_2O$ (25 ml) containing a few drops of AcOH, and dried to give the product (0.96 g, 83%).

**Z-Gln-Asn-Cys(Bzl)-Pro-Hyn(Ac)-Gly-$NH_2$ (XII)** was prepared as described for X. X (1.1 g, 1.4 mmol) and Z-Gln-ONP[6] (0.7 g, 1.8 mmol) yielded XII (1.1 g, 88%).

**Z-Phe-Gln-Asn-Cys(Bzl)Pro-Hyn(Ac)-Gly-$NH_2$ (XIII)** was synthesized by the procedure used for X, except that the evaporation of DMF was omitted. The reaction mixture turned into a semisolid mass in about 4 hr; after 20 hr it was disintegrated under THF and washed with water. From XII (1.1 g, 1.2 mmol) and Z-Phe-ONP[22] (0.6 g, 1.5 mmol), XIII (1.1 g, 92%) was obtained.

**Z-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Hyn(Ac)-Gly-$NH_2$ (XIV)** (1.2 g, 88%) was synthesized from XIII (1.1 g, 1 mmol) and Z-Tyr(Bzl)-ONP[6] (0.7 g, 1.3 mmol) as described in the preceding paragraph.

**Z-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Hyn(Ac)-Gly-$NH_2$ (XV)** was obtained by the same procedure as XIII, except that diisopropylamine[21] rather than $Et_3N$ was used for the liberation of the free amine from its hydrobromide. From XIV (0.54 g, 0.42 mmol) and Z-Cys(Bzl)-ONP[6] (0.24 g, 0.52 mmol), XV was obtained in 92% yield (0.54 g).

**Z-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Hyn-Gly-$NH_2$ (XVI).** XV (0.26 g, 0.18 mmol) was dissolved with shaking in DMF (5 ml). Anhydrous 97% $NH_2NH_2$ (0.5 ml, 15.5 mmol) was added and a two-phase mixture was formed. The mixture was stirred for 3 hr and cooled in an ice bath, and AcOH was added until acidic. The volatiles were evaporated, $H_2O$ (10 ml) was added, and the white solid was filtered, washed with water, and dried. XVI was obtained in 98% yield (0.25 g).

**ε-Hydroxynorleucine-vasopressin (I).** 1. XVI was dried at 50° *in vacuo* over $P_2O_5$ for 2 hr and left to cool overnight *in vacuo*. A solution of dried XVI (0.2 g, 0.15 mmol) in liquid $NH_3$ (about 300 ml) was reduced with Na until the blue color persisted for about 5 min. AcOH (1–2 drops) was added and the $NH_3$ was allowed to evaporate to about 20 ml. The remaining $NH_3$ was removed *in vacuo* from a frozen mixture. The residue was dissolved in 300 ml of $H_2O$, free of $O_2$, and aerated at pH 6 for 2 hr. The solution was concentrated to about 10 ml and lyophilized. The residue was dissolved in 5% AcOH and passed through a Sephadex G-25 column (50 × 2.5 cm) using 5% AcOH as the eluent. Fractions of about 6 ml were collected at a flow rate of 25 ml/hr and a single, skewed peak in fractions 20–64 (solution A) was detected by uv absorption at 280 nm. A recovery of about 90% could be deduced from the total uv absorption. Solution A was concentrated *in vacuo* to a small volume (about 10 ml) and lyophilized. The resulting powder was dissolved in the lower phase of the system *n*-BuOH–EtOH–0.1% AcOH (4:1:5) and the insoluble portion removed. The soluble portion was subjected to countercurrent distribution in a 520-tube automatic Craig apparatus with 3-ml phases. After 1000 transfers, uv absorbances at 275 nm were determined on every tenth tube. The experimental peaks were compared with theoretical ones calculated by the method of Craig and King.[23] Clearly two distinct materials were present. The contents of tubes 220–250 ($K_1 = 0.30$) and tubes 260–300 ($K_2 = 0.38$) were combined to give fractions 1 and 2, respectively. Each fraction was concentrated to a small volume and lyophilized. Fraction 1 contained 39 mg. On paper electrophoresis at pH 4.5 (pyridine–AcOH) for 2 hr at 3000 V, it traveled toward the cathode at the same rate as fraction 2. Qualitatively, fraction 2 dissolved much more readily than fraction 1.

A 20-mg sample of fraction 2 was further purified by partition chromatography[24] using the system 1-butanol–$C_6H_6$–Py–AcOH–$H_2O$ (600:200:100:1:900) and a 1 × 76 cm column. Fractions (1.5 ml) were collected and examined by the Folin–Lowry color test.[25,26] The peptide was eluted in fractions 105–149. These fractions were pooled, concentrated, and lyophilized. The material isolated had the same properties as I and was used without further purification for the bioassays.

**2.** I was also obtained from XV by reduction with Na in liquid $NH_3$ and air oxidation as above. In this experiment the countercurrent distribution curve contained three peaks. The materials isolated from peaks I and II were identical with those obtained

from experiment 1 above. The material isolated from the third peak ($K_3$ = 0.52) moved 2.5 times as fast as fraction 2 on tlc in system B. The amino acid analysis revealed the same amino acid composition as fraction 2. This compound is most likely the acetoxy derivative of I. It was not studied further.

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## 4(1*H*)-Quinolones. 2. Antimalarial Effect of Some 2-Methyl-3-(1′-alkenyl)- or -3-alkyl-4(1*H*)-quinolones†

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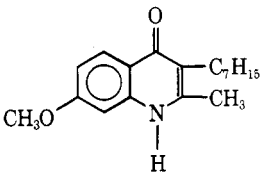
A large number of 4(1*H*)-quinolones have been prepared as intermediates in the synthesis of potential antimalarial agents of the 4-aminoquinoline series. However, except in isolated cases[1-3] the antimalarial potential of the 4-quinolones themselves have not been explored in great detail. This occurred despite the fact that the Schönhöfer theory of antimalarial action of aminoquinolines[4] would suggest that the prototropy postulated as necessary for high antimalarial activity in this class of compounds may lead *in vivo* to a 4-quinolone by hydrolysis of the 4-imino intermediate. Of the 4-quinolones reported to possess antimalarial activity, endochin (2-methyl-3-*n*-heptyl-7-methoxy-4(1*H*)-quinolone, 1)‡ was considered a very promising lead.[6] 1 was found to have excellent prophylactic as well as therapeutic activity in canaries infected with *P. praecox;* however, it was reported to have failed in clinical trials.[6]

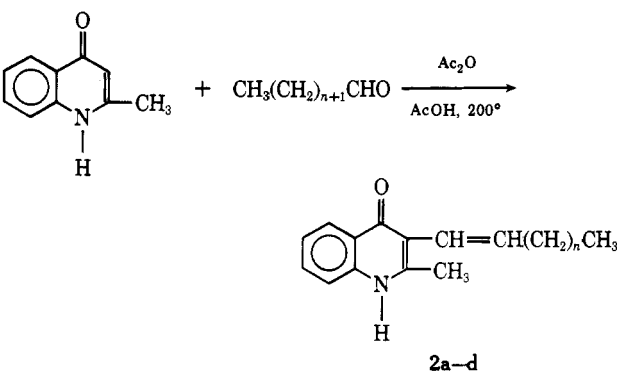


1

In connection with another investigation in progress in these laboratories, several 2-methyl-3-(1′-alkenyl)- or -3-alkyl-4(1*H*)-quinolones were prepared[7] which were structurally related to **1.** This report is concerned with the synthesis of some new 4-quinolones of the endochin type as well as their antimalarial activity.

**Chemistry.** The new 2-methyl-3-(1′-alkenyl)-4(1*H*)-quinolones (**2**) described in the present communication were prepared by the reaction of an appropriate *n*-aliphatic aldehyde with 2-methyl-4(1*H*)-quinolone in the presence of acetic anhydride and catalytic amounts of glacial acetic acid as described earlier[7] (Scheme I). The 2-methyl-3-*n*-alkyl-4(1*H*)-quinolones (**3**) were synthesized by the Conrad–Limpach method[8] which involves the condensation of aniline with an appropriately substituted β-keto ester, followed by cyclization of the 3-anilinocrotonate formed (Scheme II). 2-Methyl-3-(*N*-piperidinomethyl)-4(1*H*)-quinolone [**3h**, $R_1 = CH_2N(CH_2)_5$; $R_2 = R_3 = H$] was prepared by the method of Ghosh and Chaudhuri,[9] by treating 2-methyl-4(1*H*)-quinolone with paraformaldehyde and piperidine in a Mannich-type condensation. Compounds of type **2** and **3** are described in Table I.

**Scheme I**



**2a–d**

**Antimalarial Results.** The antimalarial results described in the present report were obtained using the *P.*

†For paper 1 in this series, see ref 7.

‡It is of interest that endochin is structurally reminiscent of the coenzyme Q and that a variety of coenzymes Q antimetabolites have been reported recently to exhibit antimalarial activity against *P. berghei* in mice. See ref 5.