Agents for Alkylating Steroid Hormone Receptors III: ω-Substituted Esters of 17α-Hydroxyprogesterone

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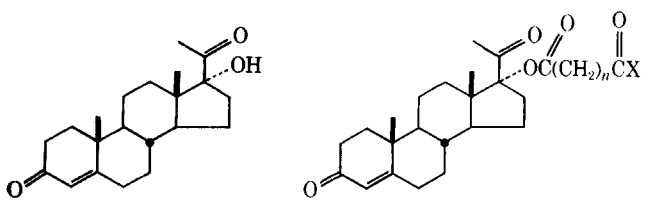
Abstract □ The synthesis and Clauberg activity of the 8-carbomethoxyoctanoate (II*e*), 9-carbomethoxynonanoate (II*f*), 9-carboethoxynonanoate (II*g*), 9-carbo-*tert*-butoxynonanoate (II*h*), 8-carboxyoctanoate (III*d*), 9-oxo-10-diazodecanoate (IV*d*), 5-oxo-6-hydroxyhexanoate (V*a*), and 6-oxo-7-hydroxyheptanoate (V*b*) esters of 17α-hydroxyprogesterone are reported and compared to the activity of related compounds.

Keyphrases □ 17α-Hydroxyprogesterone ω-substituted esters—synthesis, Clauberg activity □ Progestational activity—17α-hydroxyprogesterone-substituted esters □ Steroid hormone receptors—alkylating agents, synthesis of ω-diazoketones, activity □ NMR spectroscopy—structure □ IR spectrophotometry—structure

This study is an attempt to utilize the principles set forth by Baker (1) for the design of active-site-directed irreversible enzyme inhibitors to find compounds capable of selectively tagging the receptor sites of the steroid hormones (2, 3). Initially, analogs were chosen for the study, including, as potential alkylating agents, the ω-diazoketones derived from esters of 17α-hydroxyprogesterone (2). The results of a considerable extension of the study are reported here.

CHEMISTRY

Condensation of 17α-hydroxyprogesterone (I) with the mixed anhydrides derived from trifluoroacetic anhydride and various monoesters of dibasic aliphatic acids afforded II*a*–II*h*. Selective hydrolyses of the esters II*a*, II*b*, II*c*, and II*e* gave the carboxylic acids III*a*–III*d*. Attempted hydrolysis of II*f* and II*g*, under the conditions used to hydrolyze II*a*, II*b*, II*c*, and II*e* failed because of low solubility of II*f* and II*g* in cold methanol. Other attempts to hydrolyze selectively II*f* and II*g* with base or II*h* with trifluoroacetic acid also failed to produce significant amounts of the acid III*e*. The carboxylic acids III*a*–III*d* were converted to diazoketones IV*a*–IV*d* by the method of Wilds and Shunk (4). Diazoketones IV*a* and IV*b* were transformed to the corresponding ketols, V*a* and V*b*, by stirring their ether solutions with aqueous sulfuric acid.



I

II*a*: n = 3, X = OMe
II*b*: n = 4, X = OMe
II*c*: n = 5, X = OMe
II*d*: n = 5, X = OEt
II*e*: n = 7, X = OMe
II*f*: n = 8, X = OMe
II*g*: n = 8, X = OEt
II*h*: n = 8, X = O-*t*-Bu

III*a*: n = 3, X = OH
III*b*: n = 4, X = OH
III*c*: n = 5, X = OH
III*d*: n = 7, X = OH
III*e*: n = 8, X = OH
IV*a*: n = 3, X = CHN_2
IV*b*: n = 4, X = CHN_2
IV*c*: n = 5, X = CHN_2
IV*d*: n = 7, X = CHN_2
V*a*: n = 3, X = CH_2OH
V*b*: n = 4, X = CH_2OH

EXPERIMENTAL[1]

17α-Hydroxypregn-4-ene-3,20-dione 8-Carbomethoxyoctanoate (II*e*)—A mixture of 4.0 g. of azelaic acid monomethyl ester and 4 ml. of trifluoroacetic anhydride in 60 ml. of dry benzene was refluxed for 20 min. After cooling, 3 g. of 17α-hydroxyprogesterone and 1.0 g. of sodium carbonate were added, and reflux was resumed for 20 hr. The mixture was concentrated. The residue was diluted with ether and then extracted with aqueous sodium hydroxide. The organic phase was washed (water) and dried (magnesium sulfate), and the solvent was distilled. The residue was chromatographed over 120 g. of acid-washed alumina. Benzene eluted 2.8 g. of an oil, which crystallized from hexane to give II*e*, in a yield of 70%: m.p. 88–91°; ν(CCl_4)1738, 1720, 1688, and 1680 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.68, 1.26, 2.04, 3.67, and 5.75 corresponding to hydrogens on C-18, C-19, C-21, OMe, and C-4, respectively.

Anal.—Calc. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.46; H, 9.20.

17α-Hydroxypregn-4-ene-3,20-dione 9-Carbomethoxynonanoate (II*f*)—Monomethyl sebecate was treated with trifluoroacetic anhydride and then reacted with 17α-hydroxyprogesterone under conditions similar to those described under II*e*. Crystallization occurred from acetone–hexane to afford II*f*, in a yield of 80%: m.p. 144–145°; ν($CHCl_3$) 1730 and 1660 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.70, 1.21, 2.05, 3.71, and 5.80 corresponding to the hydrogens on C-18, C-19, C-21, OMe, and C-4, respectively.

Anal.—Calc. for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15. Found: C, 72.79; H, 9.09.

17α-Hydroxypregn-4-ene-3,20-dione 9-Carboethoxynonanoate (II*g*)—Monomethyl sebecate was treated with trifluoroacetic anhydride and then reacted with 17α-hydroxyprogesterone essentially as described under II*e*. Crystallization occurred from ether–hexane to give II*g*, in a 62% yield: m.p. 96–97°; ν (CCl_4) 1730, 1720, and 1677 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.63, 1.21, 2.10, and 5.61 and a quartet (J = 7 Hz.) at δ 4.07 corresponding to the hydrogens at C-18, C-19, C-21, C-4, and —OCH_2Me, respectively.

Anal.—Calc. for $C_{33}H_{50}O_6$: C, 73.03; H, 9.29. Found: C, 73.21; H, 9.41.

***tert*-Butyl 9-Carboethoxynonanoate (VI)**—To 8 g. of ethyl hydrogen sebecate in 200 ml. of methylene chloride was added 0.8 ml. of concentrated sulfuric acid. The mixture was cooled in an ice bath, and 200 ml. of liquid isobutylene was added. The vessel was sealed and shaken at room temperature for 20 hr. The product, VI, after purification by filtration through alumina, was obtained as an oil.

Anal.—Calc. for $C_{16}H_{30}O_4$: C, 67.10; H, 10.56. Found: C, 67.26; H, 10.68.

***tert*-Butyl 9-Carboxynonanoate (VII)**—A solution of 2 g. of VI in 50 ml. of methanol containing 0.4 g. of sodium hydroxide in 5 ml. of water was stored at 0–5° for 20 hr. The mixture was then concentrated and partitioned between ether and water. The aqueous layer was washed with ether, acidified with hydrochloric acid, and extracted with ether. The product, VII, was obtained as an oil in a yield of 780 mg.

Anal.—Calc. for $C_{14}H_{26}O_4$: C, 65.09; H, 10.14. Found: C, 65.25; H, 10.19.

17α-Hydroxypregn-4-ene-3,20-dione 9-Carbo-*tert*-butoxynonanoate (II*h*)—A solution of 0.75 g. of VI in methanol was titrated to

[1] Melting points were determined in open-capillary tubes on a Mel-Temp apparatus and are uncorrected. IR spectra were determined on a Perkin-Elmer Infracord model 137 or on a Beckman IR-8 spectrophotometer. NMR spectra were determined in $CDCl_3$ on a Varian A-60 spectrometer and are reported in parts per million downfield from a tetramethylsilane internal standard. Elemental analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Table I—Activity of 17α-Acyloxy-Substituted Progesterones in a Modified Clauberg Assay (Subcutaneous Injection)

Compound	17α-Side Chain $(CH_2)_n$	17α-Side Chain End	Total Dose, mg.	Number of Rabbits	Mean Body Weight, g. Initial	Mean Body Weight, g. Final	Mean Ovarian Weight, mg.	Mean Uterine Weight, g.	Mean Proliferation Index	Range
Progesterone			0.2	14	1084	1332	39.7	1.19	0.9^+	$0–2.0^+$
			0.5	8	1102	1357	47.2	2.26	3.1^+	$2.5–3.5^+$
IIa	3	COMe	5.0	4	1109	1260	31.5	1.18	0	0
			10.0	2	1080	1165	25.5	1.25	0.8^+	$0.5^+–1.0^+$
IIb	4	COMe	1.0	2	1136	1204	110.6	1.40	0.3^+	$0–0.5^+$
			2.0	2	1043	1042	24.4	1.18	0.8^+	$0–1.5^+$
			5.0	4	1112	1230	30.8	1.74	2.5^+	$2.0–3.0^+$
IIc	5	COMe	2.0	2	1136	1423	44.7	1.51	1.5^+	$1.0^+–2.0^+$
			5.0	2	1014	1425	41.9	2.25	3.8^+	$3.5–4.0^+$
			10.0	2	1111	1240	71.7	3.14	4.0^+	4.0^+
IIe	7	COMe	1.0	2	991	1344	35.8	1.30	0	0
			2.0	6	1178	1362	44.0	1.37	0.6^+	$0–1.5^+$
			5.0	6	1120	1458	37.4	1.92	3.4^+	$2.5^+–4.0^+$
			20.0	2	1069	1341	32.3	3.32	4.0^+	4.0^+
IIj	8	COMe	2.0	2	1284	1244	38.3	1.05	0	0
			5.0	4	1044	1321	58.1	1.46	0.9^+	$0–2.5^+$
			10.0	4	1100	1358	45.1	1.53	1.6^+	$0–3.0^+$
			15.0	2	1094	1507	24.3	1.46	2.0^+	2.0^+
			20.0	2	1036	1226	79.6	1.50	3.3^+	$3.0^+–3.5^+$
IIg	8	COEt	2.0	4	1089	1444	44.8	1.39	0.8^+	$0.5^+–1.0^+$
			5.0	4	1222	1415	50.2	2.21	3.8^+	$3.5^+–4.0^+$
			10.0	2	1067	1156	44.7	2.38	4.0^+	4.0^+
IIh	8	COtBu	5.0	2	916	1072	40.8	1.04	0	0
			10.0	2	1082	1315	41.4	1.52	0.8^+	$0.5^+–1.5^+$
IIIa	3	COH	2.0	2	1209	1511	38.6	1.10	0	0
			20.0	2	1020	1466	43.5	1.36	0.8^+	$0.5^+–1.0^+$
IIIb	4	COH	2.0	4	1078	1330	48.0	1.17	0	0
			5.0	2	1210	1389	42.7	1.40	1.0^+	1.0^+
			10.0	2	1210	1377	53.0	1.98	2.0^+	2.0^+
			20.0	2	1156	1443	58.4	1.96	2.3	$2.0–2.5^+$
IIIc	5	COH	2.0	2	1077	1068	31.8	1.53	1.0^+	1.0^+
			20.0	2	1103	1384	40.6	2.10	2.3^+	$2.0^+–2.3^+$
IIId	7	COH	1.0	2	1100	1450	47.2	1.12	0	0
			2.0	2	997	1184	36.1	0.96	0.3^+	$0–0.5^+$
			5.0	2	979	1413	52.4	1.25	0.5^+	$0–1.0^+$
			10.0	2	1175	1496	68.5	1.74	0.3^+	$0–0.5^+$
IVa	3	$CCHN_2$	1.0	2	1066	1314	32.2	1.10	0	0
			2.0	2	1072	1257	30.2	1.40	2.3^+	$2.0–2.5^+$
			5.0	4	1078	1257	36.8	1.53	2.6^+	$2.5^+–3.0^+$
			10.0	2	1141	1365	35.6	1.98	3.8^+	$3.5^+–4.0^+$
IVb	4	$CCHN_2$	1.0	2	807	1046	29.2	1.29	2.0^+	2.0^+
			2.0	2	868	1050	27.6	1.40	3.0^+	$2.5^+–3.5^+$
			5.0	2	852	1039	45.0	2.10	2.8^+	$1.5^+–4.0^+$
IVc	5	$CCHN_2$	1.0	2	1078	1333	33.6	1.12	1.0^+	1.0^+
			2.0	2	1290	1557	47.8	1.55	2.3^+	$1.5^+–3.0^+$
			5.0	2	1260	1427	38.6	2.12	3.5^+	$3.0^+–4.0^+$

Table I (*Continued*)

Compound	—17α-Side Chain— $(CH_2)_n$	End	Total Dose, mg.	Number of Rabbits	Mean Body Weight, g. Initial	Final	Mean Ovarian Weight, mg.	Mean Uterine Weight, g.	Mean Proliferation Index	Range
IV*d*	7	$\overset{O}{\overset{\|\|}{C}}CHN_2$	1.0	2	1118	1213	43.6	0.89	0	0
			2.0	4	1090	1300	35.2	1.12	0.3+	0–0.5+
			5.0	2	1276	1436	34.1	2.52	4.0+	4.0+
V*a*	3	$\overset{O}{\overset{\|\|}{C}}CH_2OH$	1.0	2	1182	1508	49.0	1.56	0	0
			5.0	2	976	1336	59.6	1.76	2.8+	2.5+–3.0+
			20.0	2	1052	1404	55.8	2.78	4.0+	4.0+
V*b*	4	$\overset{O}{\overset{\|\|}{C}}CH_2OH$	1.0	2	968	1278	39.8	1.36	0.5+	0.5+
			2.0	2	1054	1382	32.0	1.61	0.8+	0.5+–1.0+
			5.0	2	1150	1445	28.6	1.92	2.5+	2.0+–3.0+

the phenolphthalein end-point with aqueous sodium hydroxide. The resulting salt was rigorously dried. It was then suspended in 20 ml. of dry glyme, and trifluoroacetic anhydride was added (approximately 0.42 ml.) until a homogeneous solution was obtained. The solution was refluxed for 1 hr., and then 0.6 g. of I was added. After the mixture was refluxed for 20 hr., it was worked up in the usual way. Chromatography over neutral alumina afforded II*h* as crystals from hexane, in a yield of 34%: m.p. 77–79°; ν (CCl_4) 1722 and 1675 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.68, 1.21, 1.50, and 2.03 corresponding to the hydrogens at C-18, C-19, O-*tert*-Bu, and C-21, respectively.

Anal.—Calc. for $C_{35}H_{54}O_6$: C, 73.65; H, 9.54. Found: C, 73.47; H, 9.35.

17α-Hydroxypregn-4-ene-3,20-dione 8-Carboxyoctanoate (III*d*)—A solution of II*e* in 50 ml. of methanol containing 5 ml. of 2 *N* sodium hydroxide was kept under nitrogen at 0–5° for 24 hr. The methanol was distilled under reduced pressure, and the residue was partitioned between ether and water. The aqueous layer was acidified and extracted with ether. The latter extract was washed with water, dried with magnesium sulfate, and concentrated to leave a residue which crystallized from ether–hexane to give III*d*, in a 42% yield: m.p. 140–141°; ν ($CHCl_3$) 3250, 1720, 1710, 1700, and 1660 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.69, 1.21, 2.04, and 5.76 corresponding to the hydrogens at C-18, C-19, C-21, and C-4, respectively.

Anal.—Calc. for $C_{30}H_{44}O_6$: C, 71.97; H, 8.86. Found: C, 72.15; H, 9.05.

17α-Hydroxypregn-4-ene-3,20-dione 4-Oxo-10-diazodecanoate (IV*d*)—A solution of 400 mg. of III*d* in methanol was titrated to the phenolphthalein end-point with aqueous sodium hydroxide. The resulting salt was dried and then slurried in 15 ml. of dry benzene. Three milliliters of pyridine was added. The mixture was cooled in an ice bath, and 4 ml. of oxalyl chloride was added. After stirring for 30 min. in the cold, the mixture was concentrated under reduced pressure. The residue was three times mixed with 10-ml. portions of dry benzene and distilled to dryness under reduced pressure. After the residue was dried at high vacuum, it was dissolved in 10 ml. of benzene and filtered. The filtrate was added with cooling to an ethereal, alcohol-free solution of diazomethane, which had previously been prepared from 10 mmoles of *N*-nitroso-*N*-methyl-*p*-toluenesulfonamide. The solvent was removed under vacuum, and the residue was crystallized from ether–hexane to give IV*d*, in a yield of 62%: m.p. 60–62.5°; ν (CCl_4) 2100, 1720, and 1675 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.68, 1.20, 2.03, 5.29, and 5.87 corresponding to the hydrogens on C-18, C-19, C-21, $\overline{C}HN_2$, and C-4, respectively.

Anal.—Calc. for $C_{31}H_{44}N_2O_5$: C, 70.96; H, 8.54; N, 5.34. Found: C, 71.07; H, 8.54; N, 5.11.

17α-Hydroxypregn-4-ene-3,20-dione 5-Oxo-6-hydroxyhexanoate (V*a*)—To a solution of 150 mg. of IV*a* in 20 ml. of ether and a few drops of methylene chloride was added a solution of 0.5 ml. of concentrated sulfuric acid in 10 ml. of water. The mixture was stirred at room temperature for 48 hr. and was then diluted with ether, extracted with aqueous sodium bicarbonate, washed with water, and dried. The solvent was distilled. The residue crystallized from hexane–acetone to afford V*a*, in a 56% yield: m.p. 114–115°; ν ($CHCl_3$) 3500, 1715, and 1660 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.68, 1.21, 2.07, 4.22, and 5.78 corresponding to the hydrogens at C-18, C-19, C-21, C(=O)$\overline{C}H_2$OH, and C-4, respectively.

Anal.—Calc. for $C_{27}H_{38}O_6$: C, 70.72; H, 8.35. Found: C, 70.53; H, 8.52.

17α-Hydroxypregn-4-ene-3,20-dione 6-Oxo-7-hydroxyheptanoate (V*b*)—A solution of 110 mg. of IV*b* in ether was treated under essentially the same conditions as described for V*a*. The product, V*b*, crystallized from ether–hexane, in a yield of 84%: m.p. 100–104° dec.; ν ($CHCl_3$) 3500, 1720, and 1660 cm.$^{-1}$. The NMR spectrum had singlets at δ 0.68, 1.21, 2.07, 4.22, and 5.78 corresponding to the hydrogens at C-18, C-19, C-21, C(=O)$\overline{C}H_2$OH, and C-4, respectively.

Anal.—Calc. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found: C, 71.07; H, 8.66.

RESULTS AND DISCUSSION

As shown in Table I, the esters II*a*–II*c* and II*e*–II*h* were tested by the McPhail modification of the Clauberg assay[2]. The progestational activity increased with chain length for the glutarate ester II*a* through the pimelate ester II*c*. It then decreased slightly for the azelate ester II*e* and decreased further for the methyl and *tert*-butyl sebacates II*f* and II*h*. However, the activity of the ethyl sebacate II*g* appears to be at least as great as that of the methyl adipate II*b* or of the methyl azelate II*e*. This anomaly appears to rule out the possibility of correlating the variation in activity of these esters solely with a single empirical parameter, such as Hansch's π (5). The acid III*d* and the diazoketone IV*d*, which have more than seven contiguous carbon atoms in their side chains, also are less active than would be expected on the basis of the activities noted for lower members of the respective homologous series.

n-Alkyl esters of 17α-hydroxyprogesterone show maximal Clauberg activity when six or seven contiguous carbon atoms are present in the side chain and then show a progressive decrease both in intensity and in duration of Clauberg activity as the acid moiety of the esters is homologated (6). A number of β-(*p*-alkoxyphenyl)-propionate esters of 17α-hydroxyprogesterone are reported to retain high Clauberg activity (7), but the ether oxygen in these compounds occupies essentially the position that would be occupied by the eighth carbon in a straight-chain ester. This results both in an increase in hydrophilic character and a bend in the molecule at that position.

The reported data are in accord with the view that straight-chain esters of 17α-hydroxyprogesterone, which have eight or more contiguous carbon atoms in the side chains, undergo an additional interaction with some biological receptor.

The diazoketones IV*a*–IV*d* are all at least as active as (in some cases, more active than) the esters from which they are derived.

[2] Clauberg assays were performed at the Endocrine Laboratories, Madison, Wis.

Of particular significance is the fact that diazoketone IV*b* is more active than ketol V*b* which is derived from it. This strongly supports the assumption that the Clauberg activity measured for the diazoketones is a measure of their inherent activity and not simply a measure of the activity of their hydrolysis products.

It was also of interest to observe that the carboxylic acids III*a*–III*d* showed a low, but real, Clauberg activity. The limited data available suggest that although these acids may not elicit a high level of response in this assay, up to a response level of 2.0+, their activity is equal to, or slightly greater than, that of the isomeric methyl esters. The calculated π-values (5) are also slightly greater for the acids than for the isomeric methyl esters.

None of the diazoketones has an activity so abnormal, either in comparison with the other members of this series or with that of the analogous methyl esters, as to suggest that it has alkylated the Clauberg receptor. However, the activity of diazoketones IV*a*, IV*b*, and, to a lesser extent, IV*c* seems surprisingly high, and it may warrant a reinvestigation with an assay based on intrauterine application of these compounds.

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Spectrophotofluorometric Determination of Salicylamide in Blood Serum and Urine

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Abstract □ A spectrophotofluorometric method is reported for the determination of salicylamide in blood serum and urine. The procedure involves the simultaneous determination of salicylamide and salicylic acid at pH 11 after the acid hydrolysis of the salicylamide metabolites. The precision and accuracy of the method are comparable to the regular ferric-ion complex procedure. The present method has the sensitivity and specificity desired in the assay of blood serum and urinary salicylamide concentrations after the oral ingestion of a 325-mg. dose of the drug. The method may be used in monitoring bioavailability of salicylamide provided by different dosage forms.

Keyphrases □ Salicylamide—spectrophotofluorometric determination, in blood serum, urine □ Bioavailability evaluation—application of spectrophotofluorometric salicylamide determination □ Spectrophotofluorometry—salicylamide determination, blood serum, urine

The absorption and pharmacokinetics of salicylamide in humans were found to be both dose and dosage form dependent (1–3). However, most of the salicylamide blood level results reported were elicited with larger than normal doses of the drug. Therefore, a sensitive yet specific analytical method is needed to study the blood serum levels of salicylamide in humans after the ingestion of as little as 325 mg. of the drug. The ferric-ion complex colorimetric method is not sufficiently sensitive for this purpose.

In basic solution, salicylamide is fluorescent. Based on this property, several methods were reported for the assay of free salicylamide in biological fluids. Lange *et al.* (4) employed a fluorometric technique to determine salicylamide and other salicylates following a gel filtration separation. Barr and Riegelman (3) detailed a spectrophotofluorometric procedure for the determination of salicylamide and its metabolites in plasma and other biological fluids. Unfortunately, the body metabolizes salicylamide; hence, very little exists as free salicylamide in the blood or urine (1, 5, 6). For this reason, total salicylamide is determined as free salicylamide after acid hydrolysis. Barr and Riegelman (3)

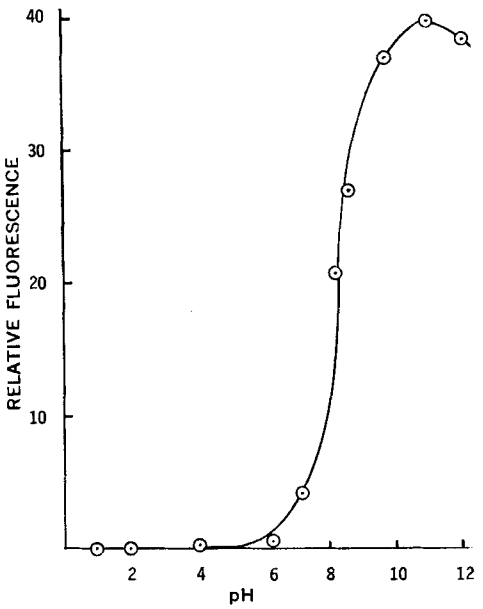

Figure 1—*Relative fluorescence of 0.1 mcg./ml. of salicylamide as a function of pH at the activation and emission wavelengths of 340 and 435 nm., respectively.*