[CONTRIBUTION FROM THE RESEARCH DEPARTMENT OF CIBA PHARMACEUTICAL PRODUCTS, INC.]

Rauwolfia Alkaloids. XXXII.[1] Further Studies on the Synthesis and Activity of Reserpine Analogs

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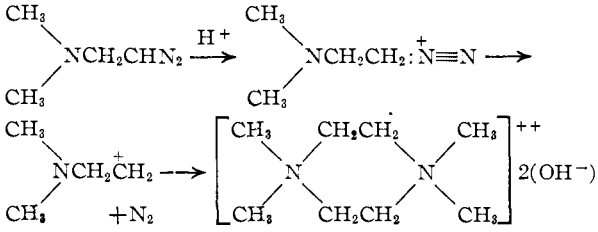
In a continuing study of reserpine analogs new derivatives of reserpic acid of biological interest were synthesized. The previously unreported O-(3,4,5-trimethoxybenzoyl)-reserpic acid was prepared. In the course of the syntheses use was made of several novel diazoalkanes.

The study of the synthesis and biological activity of a large number of esters of methyl reserpate[1] gave encouragement to a continued program seeking reserpine analogs of enhanced activity, either sedative or hypotensive. In this connection novel esters of reserpic acid were prepared by: (a) reaction of reserpic acid with diazoalkanes and (b) the base-catalyzed reaction of reserpic acid lactone with alcohols.

(a) Diazoalkanes were prepared from the corresponding ethyl carbamates. The relatively nonvolatile diazoalkanes obtained directly by treatment of the carbamates with alkali were not distilled but were used in the form of crude ether extracts. As expected the longer diazoalkanes reacted much more slowly and gave lower yields than diazomethane. Diazotoluene gave no appreciable reaction with reserpic acid. 2-Dimethylamino-diazoethane was obtained with difficulty and yields of ester were erratic, probably due to rapid decomposition of the diazo compound.[2] It seems likely that 2-dimethylamino-diazoethane would dimerize easily to the piperazinic base



(b) Temperature and time of reaction were found to be critical in the base-catalyzed reaction of reserpic acid lactone with various alcohols. With benzyl alcohol best yields were obtained by heating at 90–105° for not more than 1–2 hours using trace amounts of sodium alkoxide catalyst. At higher temperatures and longer reaction times considerable decomposition occurred and made it difficult to isolate any product.

Acylation of reserpic acid esters with acyl chlorides was carried out in pyridine solution at room temperature. It was discovered that more granular crude products were obtained by pouring the acylation mixtures into cold water containing a small amount of ammonia.

The long sought and previously unreported 0-(3,4,5-trimethoxybenzoyl)-reserpic acid was prepared by acylation of benzyl reserpate with 3,4,5-trimethoxybenzoyl chloride followed by removal of the benzyl group by hydrogenation over palladium-in-methanol. That the compound possessed the same stereochemistry as reserpine was confirmed by conversion with diazomethane to reserpine.

Compared with reserpine the biological activity of 0-(3,4,5-trimethoxybenzoyl)-reserpic acid is very low thus indicating that both the 16- and 18-positions of reserpic acid must be substituted in order to elicit reserpine-like activity since it has been shown previously that methyl reserpate[1] has only slight biological activity.[3]

Some interesting observations may be made concerning biological activity of the new esters reported here. When a benzoyl ester carries methoxy groups in the 3,5-positions, substitution in the 4-position markedly enhances the activity. Introduction of 2-methoxyethyl or 2-dimethylaminoethyl groups in the 16-position appears to enhance reserpine-like sedative activity and minimize hypotensive activity. Higher 16-alkyl esters showed diminished reserpine-like activity.

Of the compounds prepared related to syrosingopine (methyl 0-(3,5-dimethoxy-4-ethoxyformyloxybenzoyl)-reserpate)[1] some approached but none exhibited as favorable a ratio of hypotensive to sedative activity. Indeed where syrosingopine has good hypotensive activity without sedation, substitution of 2-methoxyethyl in the 16-position tends to reverse the effect and the resultant compound shows marked sedative activity.

Acknowledgments.—We wish to express our sincere appreciation to Dr. E. Schlittler for his interest and encouragment. We should like to thank Mr. L. Dorfman and his associates for the microanalytical data. It is a special pleasure to acknowledge the close and valuable coöperation of Dr. A. Plummer, Dr. W. Barrett and their associates in our Macrobiology Division who were responsible for the biological evaluation of our substances.

Experimental

Melting Points.—Melting points are corrected and were determined in capillary tubes in an electrically heated aluminum block.

Reserpic acid, methyl reserpate and ethyl reserpate were prepared according to published procedures.[4]

Preparation of 16-Substituted Esters of Reserpic Acid.—The methods of preparation of compounds listed in Table I are indicated below: (A) This procedure is illustrated by the

(1) Part XXXI, The Synthesis and Activity of Some Reserpine Analogs, THIS JOURNAL, **81**, 1928 (1959).

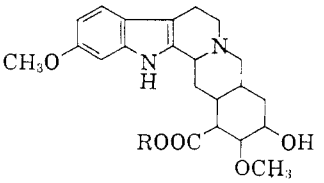
(2) We are indebted to Dr. W. A. Rosen of the CIBA Developmental Division for pointing out an analogous reaction encountered in an attempted alkylation with the tosylate of dimethylaminoethanol (W. B. Rosen, V. P. Toohey and A. C. Shabica, THIS JOURNAL, **79**, 3170 (1957)).

(3) Higher alkyl esters of reserpic acid also exhibit only minimal biological activity.

(4) L. Dorfman, *et al.*, *Helv. Chim. Acta*, **37**, 59 (1954).

TABLE I

ESTERS OF RESERPIC ACID

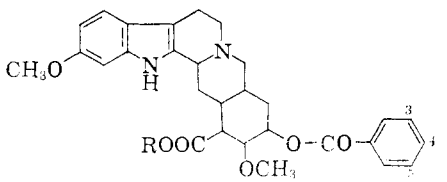


R	Method of prepn.	Yield, %	M.p., °C.	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
$CH_3CH_2CH_2$	A[a]	73	164–166	$C_{25}H_{34}N_2O_5$	67.85	66.99	7.75	7.86	6.33	6.40
$CH_3(CH_2)_2CH_2$	A	43	119–121	$C_{26}H_{36}N_2O_5$	68.39	67.85	7.95	8.03	6.14	5.79
$CH_3(CH_2)_4CH_2$	A[b]	33	115–118	$C_{28}H_{40}N_2O_5$	69.39	69.13	8.32	8.42	5.78	5.97
$C_6H_5CH_2$	B	45	202–204	$C_{29}H_{34}N_2O_5$	71.00	71.21	6.99	7.11	5.71	5.58
$(CH_3)_2NCH_2CH_2$	A[c]	14	110–114	$C_{26}H_{37}N_3O_5$	66.22	66.02	7.91	8.11	8.91	8.89
$CH_3OCH_2CH_2$	A[d,e]	40	185–186	$C_{25}H_{34}N_2O_6$	65.48	65.27	7.47	7.86	6.11	5.77

[a] May also be prepared from reserpic acid lactone by base-catalyzed reaction with propanol. [b] Ethyl N-hexylcarbamate was prepared by a procedure similar to that described in "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., N. Y., New York, N. Y., 1950, p. 278. It boiled 126–128° at 17–20 mm. It was nitrosated by a procedure analogous to that given, *ibid.*, p. 464. Diazohexane was not distilled, but the ether layer containing it was added directly to the slurry of reserpic acid. [c] Ethyl N-(2-dimethylaminoethyl)-carbamate was prepared as in *b* and nitrosated in methylene chloride solution at 0–5°, with nitrosyl chloride. Ethyl N-(2-dimethylaminoethyl)-N-nitrosocarbamate hydrochloride precipitated from the methylene chloride solution, m.p. 133–135°. Calcd. for $C_7H_{16}N_3O_3Cl$: C, 37.25; H, 7.15; N, 18.62; Cl, 15.71. Found: C, 37.42; H, 7.15; N, 18.65; Cl, 15.97. 2-Dimethylamino-diazoethane was prepared from the nitrosocarbamate. [d] Ethyl N-(2-methoxyethyl)-carbamate, b.p. 105–106° at 17–20 mm., was prepared, nitrosated and converted to 2-methoxydiazoethane as in *b*. [e] Also prepared as in procedure B, but with 17-hour reaction time.

TABLE II

DERIVATIVES OF 18-O-BENZOYLRESERPIC ACID



R	Benzoyl substituents	Method of prepn.	M.p., °C.	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
H	3,4,5-$(OCH_3)_3$	A	211–215	$C_{32}H_{38}N_2O_9$	63.25[a]	63.03	6.76[a]	6.59	4.47[a]	4.52
$(CH_3)_2NCH_2CH_2$	3,4,5-$(OCH_3)_3$	B	140–145	$C_{36}H_{47}N_3O_9$	64.94	64.74	7.12	7.14	6.31	6.14
$CH_3OCH_2CH_2$	3,4,5-$(OCH_3)_3$	B	130–134	$C_{35}H_{44}N_2O_{10}$	64.39	64.33	6.80	7.04	4.29	4.22
$CH_3CH_2CH_2$	3,4,5-$(OCH_3)_3$	B	200–201	$C_{35}H_{44}N_2O_9$	66.02	65.93	6.96	7.04	4.40	4.51
$CH_3(CH_2)_2CH_2$	3,4,5-$(OCH_3)_3$	B	192–193	$C_{36}H_{46}N_2O_9$	66.41	66.45	7.13	7.25	4.31	4.48
$CH_3(CH_2)_4CH_2$	3,4,5-$(OCH_3)_3$	B	115–120	$C_{38}H_{50}N_2O_9$	67.23	66.98	7.42	7.29	4.13	4.19
$C_6H_5CH_2$	3,4,5-$(OCH_3)_3$	B	190–192	$C_{39}H_{44}N_2O_9$	68.40	68.35	6.48	6.53	4.09	4.12
CH_3	3,5-$(OCH_3)_2$	B	239–241	$C_{32}H_{38}N_2O_8$	66.43	66.28	6.62	6.65	4.84	4.77
CH_3CH_2	3,5-$(OCH_3)_2$, 4-$OCO_2C_2H_5$	C	154–157	$C_{36}H_{44}N_2O_{11}$	63.51	63.13	6.52	6.73	4.12	4.29
$CH_3OCH_2CH_2$	3,5-$(OCH_3)_2$, 4-$OCO_2C_2H_5$	C	214–216	$C_{37}H_{46}N_2O_{12}$	62.52	62.73	6.52	6.62	3.94	4.13
$CH_3CH_2CH_2$	3,5-$(OCH_3)_2$, 4-$OCO_2C_2H_5$	C	141–145	$C_{37}H_{46}N_2O_{11}$	63.96	64.08	6.67	6.69	4.03	3.98
$CH_3(CH_2)_2CH_2$	3,5-$(OCH_3)_2$, 4-$OCO_2C_2H_5$	C	137–140	$C_{38}H_{48}N_2O_{11}$	64.39	64.68	6.82	6.95	3.95	3.92
$CH_3(CH_2)_4CH_2$	3,5-$(OCH_3)_2$, 4-$OCO_2C_2H_5$	C	125–128	$C_{40}H_{52}N_2O_{11}$	65.19	65.10	7.11	7.08	3.80	3.94
CH_3CH_2	3-$N(CH_3)_2$	B	229–230	$C_{33}H_{41}N_3O_6$	68.85	68.55	7.18	7.03	7.30	7.32
$CH_3OCH_2CH_2$	3-$N(CH_3)_2$	B	130–135	$C_{34}H_{43}N_3O_7$	67.42	67.13	7.16	7.18	6.94	6.91

[a] Calculated with one mole of methanol of crystallization.

preparation of propyl reserpate. Diazopropane prepared[5] from 64 g. of ethyl N-propyl-N-nitrosocarbamate was distilled directly into a slurry of 20.5 g. of reserpic acid in 40 ml. of a 1:1 mixture of chloroform and ethanol. After several hours the excess diazopropane was decomposed by the addition of a few drops of acetic acid and the solvent evaporated *in vacuo*. To the residue was added 400 ml. of water and 20 ml. of aqueous ammonia. This mixture was extracted with 200 ml. and 100 ml. of methylene chloride and the combined extracts filtered through a short column of Florisil. After evaporation of the solvent propyl reserpate was recrystallized from ethyl acetate–petroleum ether.

(5) E. I. "Beilstein," Vol. 1, p. 334.

(B) Benzyl **Reserpate**.—To a solution of 50 mg. of metallic sodium in 65 ml. of benzyl alcohol was added 7.8 g. of reserpic acid lactone.[4] The mixture was heated at 85–90° for 20 minutes, which effected dissolution of all solid, and then allowed to cool slowly to room temperature. The solution was acidified with 1:1 HCl and poured into 700 ml. of ether. The supernatant solvent was decanted from the precipitated gum which was redissolved in a small amount of methanol and again precipitated with 700 ml. of ether. The ether was decanted and the gum dissolved in 150 ml. of water. The solution was made basic with excess ammonia and extracted several times with ethyl acetate. The combined ethyl acetate extracts were filtered through a short column of Florisil, evaporated *in vacuo* and the

foamy residue was recrystallized from ethyl acetate to yield benzyl reserpate, m.p. 202–204°.

Preparation of Derivatives of 18-O-Benzoylreserpic Acid.—The compounds listed in Table II were prepared by the following methods: (A) Benzyl 0-(3,4,5-trimethoxybenzoyl)-reserpate (1.0 g.) was suspended in 400 ml. of methanol and hydrogenated over 500 mg. of palladium black at atmospheric pressure for 4 hr. Most of the hydrogen was absorbed rapidly during the first half-hour. The mixture was then diluted with 100 ml. of methylene chloride to dissolve crystalline products, filtered and the filtrate evaporated *in vacuo*. The residue was dissolved in methylene chloride, a small amount of methanol added and the solution evaporated carefully under nitrogen on the steam-bath to crystallize the desired 0-(3,4,5-trimethoxybenzoyl)-reserpic acid. After recrystallization the yield was 870 mg.

Proof of constitution was afforded by reaction of 45 mg. of the substance in methylene chloride–methanol–ether with excess of diazomethane overnight to yield 40 mg. of a compound, m.p. 266–271°, identical by infrared spectra with an authentic sample of reserpine.

Anal. Calcd. for $C_{33}H_{40}N_2O_9$: C, 65.11; H, 6.62; N, 4.60. Found: C, 64.91; H, 6.61; N, 4.70.

(B) The general procedure followed using the corresponding acyl halides and reserpic acid esters is illustrated by the preparation of 2-dimethylaminoethyl 0-(3,4,5-trimethoxybenzoyl)-reserpate.

To 2.37 g. (0.005 mole) of 2-dimethylaminoethyl reserpate, dried *in vacuo* at 85–90° for 2 hr., was added 30 ml. of dry pyridine and 1.38 g. (0.006 mole, 20% excess) of 3,4,5-trimethoxybenzoyl chloride. The mixture was cooled under tap water with constant shaking for a period of 5 minutes and allowed to stand at room temperature overnight. The pyridine solution was poured into 300 ml. of ice-cold water containing 10 ml. of ammonium hydroxide. Precipitated solid, which became granular on standing, was filtered, washed with water and air-dried. The crude ester, dissolved in methylene chloride, was filtered through a short Florisil column and recrystallized from ethyl acetate–petroleum ether; yield 1.5 g.

(C) The procedure was the same as B except that the reaction mixture was poured into water and carefully adjusted with ammonia to approximately *p*H 8–9. An excess of ammonia was avoided in order to minimize possible ammonolysis of the ethoxyformyloxy group.

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[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY]

The Association of Nickel(II) Ion with Peptides[1]

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In solutions of peptides such as glycylglycine and nickel(II) ion with a ligand-to-metal ratio greater than two to one, two equivalents of acid, in addition to that found when the peptide is titrated in the absence of Ni(II), are titrated at about *p*H 9. Since titration of the corresponding solutions of glycylsarcosine and nickel(II) give no indication of additional equivalents in this region, the amide hydrogen is implicated as the source of the protons, as has previously been demonstrated for copper(II) complexes of peptides. Complexes of nickel(II) with glycinamide, triglycine and tetraglycine, which are blue in relatively acid solutions, yield yellow solutions on ionization of the amide hydrogens. Absorption maxima for the three compounds named above were at 438, 430 and 412 mμ, respectively, with molar extinction coefficients of 61, 240 and 215. For tetraglycine the titration curve of the three amide hydrogens showed extremely high buffer capacity; the three *pK* values were closer together than would be expected statistically for equivalent and independent groups, and an appreciable time was required for equilibration during titration. The resulting complex contains one mole of tetraglycine per mole of Ni(II). The color change presumably indicates a transition from an octahedral to a planar configuration.

Heretofore, the association of divalent copper with peptides has been regarded as unique, in that Cu(II) was the only metal ion which was known to induce ionization of an amide hydrogen after combination with the amino group of a peptide such as glycylglycine. Convincing evidence has been presented that it is the amide hydrogen and not a proton from the hydration sphere of the cupric(II) ion that ionizes at about *p*H 4.[2–5] The ionization of the amide hydrogen apparently does not occur for simple peptide complexes of divalent cobalt,[3] manganese,[3,4] magnesium[4] and zinc.[6] In this paper evidence is presented that divalent nickel complexes are also effective in promoting the ionization of amide hydrogens.

Experimental

Standardized sodium hydroxide solution was added from a Gilmont ultramicroburet of 1.00-ml. capacity to solutions of nickel nitrate, ligand and sufficient KNO_3 to yield an ionic strength of about 0.16. The temperature was 25°. The distilled water used was passed through a mixed bed ion-exchange column before use. All the peptides were obtained commercially except for the glycylsarcosine which was a gift from W. L. Koltun and R. Roth. The *p*H was measured on a Beckman Model G *p*H meter. The spectra were recorded on a Beckman Model DU spectrophotometer, and for this purpose the nickel was added as the chloride.

Results

The titration curves for solutions of varying ratios of glycylglycine and divalent nickel ion are shown in Fig. 1. For all ratios greater than two—precipitation occurs at lower ratios—two additional equivalents are titrated in the 9 to 11 *p*H region. There is some evidence that a third additional equivalent is titrated at high ratios with a pK_3 of about 10.7. The titration curves for similar mixtures of glycylsarcosine and nickel are also

(1) This work was supported by grants from the United States Public Health Service (H-3169) and from the National Science Foundation (G-3230). Some of the work reported here is presented in the honors thesis of Michael Chamberlin in Biochemical Sciences, Harvard, 1959.

(2) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246, 257 (1955).

(3) S. P. Datta and B. R. Rabin, *Biochim. Biophys. Acta*, **19**, 572 (1956); *Trans. Faraday Soc.*, **52**, 1117, 1123 (1956); B. R. Rabin, *ibid.*, **52**, 1130 (1956); *Biochem. Soc. Symposia*, **15**, 21 (1958).

(4) C. B. Murphy and A. E. Martell, *J. Biol. Chem.*, **226**, 37 (1957).

(5) W. L. Koltun and F. R. N. Gurd, THIS JOURNAL, **81**, 301 (1959); W. L. Koltun, M. Fried and F. R. N. Gurd, *ibid.*, **82**, 233 (1960).

(6) F. R. N. Gurd, private communication. M. Fried and F. R. N. Gurd (private communication) have independently observed the titration of additional hydrogens in solutions of glycylglycine and divalent nickel. A. R. Manyak, C. B. Murphy and A. E. Martell, *Arch. Biochem. and Biophys.*, **59**, 373 (1955), observed the titration of one additional hydrogen in a 2:1 mixture of glycylglycine and nickel(II) ion. However, their evidence that the peptide hydrogen is titrated, as opposed to a proton from the hydration sphere of the metal ion, is not conclusive and even questionable as they also claim peptide hydrogen ionization for the cobalt(II) complex which is apparently not the case.[3]