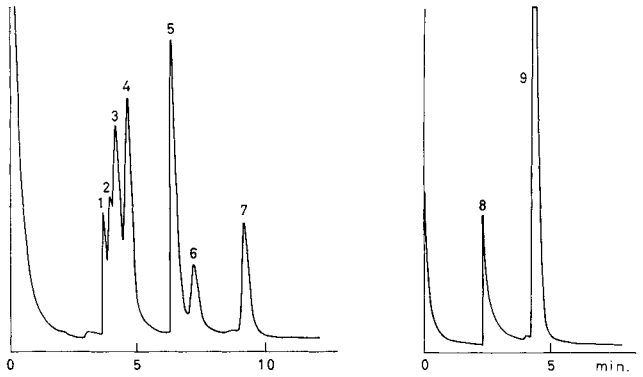

Figure 1—*Gas chromatogram of (left) phenolic; (right) nonphenolic alkaloid fraction of* Echinocereus merkeri. *Column: 1.83 m. (6 ft.) × 0.41 cm. (0.125 in.); 5% SE-30 on Gas Chrom P; initial temp. 130°, progr. 6°/min. Peak 1 = hordenine, peak 2 = 3-methoxytyramine, peak 3 = unknown, peak 4 = N,N-dimethyl-3,4-dimethoxyphenethylamine, peak 5 = salsoline, peaks 6–7 = unknowns, peak 8 = unknown (M^+ = m/e 140, base peak = 58), peak 9 = mixture of* N-*methyl- and* N,N-*dimethyl-3,4-dimethoxyphenethylamine.*

acc. to Brockmann) and eluted with the solvents shown in Table I. The eluates were investigated by TLC and GLC using SE-30 and XE-60 columns (1). *N,N*-Dimethyl-3,4-dimethoxyphenethylamine, and *N*-methyl-3,4-dimethoxyphenethylamine, were isolated using preparative column chromatography, and recrystallized as hydrochlorides from hot chloroform upon the dropwise addition of ether. Melting points of hydrochlorides, VII·HCl 193–196°, VI·HCl 134–136°. Reported (1) m.p.'s for these compounds are 194–197° and 196–197°, respectively.

Mass spectra were recorded with a gas chromatograph mass spectrometer (LKB9000), ion source 270°, electron energy 70 eV., and ionization current 60 μamp.

Mass spectrometric data; compounds IV-V, see *Reference* 4. *N*-Methyl-3,4-dimethoxyphenethylamine, major peaks, *m/e* 44 (100%), 151 (3%), 152 (40%), 195 (M^+ 1%). *N,N*-Dimethyl-3,4-dimethoxyphenethylamine, major peaks, *m/e* 58 (100%), 151 (3%), 152 (1%), 209 (M^+, 1%). Salsoline, major peaks, *m/e* 149 (3%), 153 (7%), 154 (4%), 178 (100%), 192 (6%), 193 (M^+, 3%).

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Ring Opening of Cyclic Salicylamides

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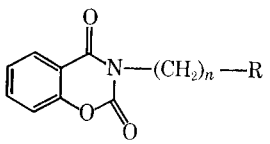
Abstract □ The ring opening of four cyclic salicylamides to their phenolic analogs is described. Compounds in which a basic group is separated from the amide nitrogen by a three-carbon chain were found to undergo this reaction considerably more slowly than compounds with a two-carbon chain. Ring opening was shown to be pH dependent and is base-catalyzed.

Keyphrases □ Salicylamides, cyclic—ring opening □ Phenolic salicylamides—synthesis □ pH effect—ring opening, salicylamides □ Radiometric determination—ring opening, salicylamides □ Colorimetric analysis—spectrophotometer

Because of the presence of the cyclic acyl urethan group and the consequent opportunity for nucleophilic attack on the carbonyl group, cyclic salicylamides of the type respresented by the generic structure



have been considered unstable in the presence of a base (1). The present report describes experiments undertaken in an effort to obtain a cyclic salicylamide which would be stable under physiological conditions and a study of the pH dependent nature of the conversion of a cyclic salicylamide to its phenolic analog.

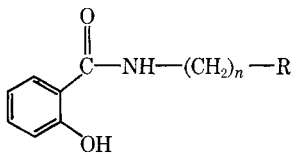
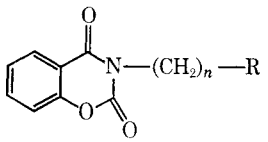
METHODS AND MATERIALS

Chemical Synthesis—Four pairs of phenolic salicylamides and the corresponding cylic compounds (Table I) were prepared, the phenolic compounds by a single method, and the cyclic compounds by one of two methods [these methods represent modifications of the procedure described by Shapiro *et al.* (2)]; representative syntheses are described.

N-(2-Morpholinoethyl)salicylamide hydrochloride (III) was prepared by heating a mixture of 60.8 g. (0.40 mole) of methyl salicylate and 52.0 g. (0.40 mole) of *N*-(2-aminoethyl)morpholine under reflux for 18 hr. The resulting methanol was removed by distillation over a period of 4 hr. and the remaining material was then distilled under reduced pressure. A 63.5-g. fraction was collected between 180–190° at 0.1 mm.; the IR spectrum[1] showed an amide carbonyl absorption at 1650 $cm.^{-1}$. The hydrochloride was prepared by adding 50 ml. of a 2.2 *N* HCl solution in 2-propanol to 22.5 g. (0.09 mole) of the free base. Upon addition of ether, a white solid formed which was recrystallized from methanol–ether.

[1] IR spectra were obtained with a Perkin-Elmer model 237 grating spectrophotometer.

Table I—Structures of, and Analytical Values for, the Salicylamides

Compd.	*n*	R	M.p., °C.[a]	Empirical Formula	Anal., % Calcd.	Found
						
I[b]	2	$N(C_2H_5)_2$	114–115	$C_{13}H_{20}N_2O_2 \cdot C_4H_4O_4$	N (total), 7.95 N (basic), 3.97	N (total), 7.87 N (basic), 3.97
II[c]	3	$N(C_2H_5)_2$	160–162	$C_{14}H_{22}N_2O_2 \cdot C_2H_2O_4$	C, 56.45 H, 7.11 N (total), 8.23	C, 55.42 H, 7.13 N (total), 8.18
III[d]	2		245–247	$C_{13}H_{18}N_2O_3 \cdot HCl$	C, 54.44 H, 6.68 N (total), 9.78	C, 54.27 H, 6.88 N (total), 9.56
IV[d]	3		80–82	$C_{14}H_{20}N_2O_3$	N (total), 10.60 N (basic), 5.30	N (total), 10.54 N (basic), 5.31
				52940T		
						
V[e]	2	$N(C_2H_5)_2$	232–233	$C_{14}H_{18}N_2O_3 \cdot HCl$	C, 56.28 H, 6.41 N (total), 9.35	C, 56.77 H, 6.65 N (total), 9.33
VI	3	$N(C_2H_5)_2$	174–176	$C_{15}H_{20}N_2O_3 \cdot HCl$	C, 57.62 H, 6.77 N (total), 8.96	C, 57.30 H, 7.11 N (total), 8.83
VII[e]	2		253–255	$C_{14}H_{16}N_2O_4 \cdot HC$	C, 53.74 H, 5.48 N (total) 8.96	C, 53.77 H, 5.66 N (total) 8.97
VIII	3		227–228	$C_{15}H_{18}N_2O_4 \cdot HCl$	N (total), 8.57 N (basic), 4.28	N (total) 8.67 N (basic) 4.26

[a] Uncorrected. [b] Synthesis of free base described by Massarani (4). [c] Analysis for neutral equivalent; calcd.—113.5, found—113.9; synthesis of hydrochloride described by Dengel (5). [d] Synthesis of free base described by Horrom and Swett (6). [e] Synthesis of free base described by Robapharm (7).

Compounds I,[2] II, and IV were synthesized in an analogous fashion.

3-(2-Morpholinoethyl)-2H-1,3-benzoxazine-2,4-(3H)-dione hydrochloride (VII) was prepared by the addition, while stirring at 0–5°, of 18.5 g. (0.164 mole) of ethyl chloroformate to 41 g. (0.164 mole) of *N*-(2-morpholinoethyl)salicylamide in 100 ml. of pyridine and 60 ml. of acetonitrile. After the addition was completed, the solvents were removed by distillation at atmospheric pressure. When the internal temperature reached 120°, the thick residue was diluted with 2-propanol, and the slurry was cooled in an ice bath. The resulting hydrochloride was recrystallized from methanol–ether, resulting in a yield of 22.0 g.; the IR spectrum showed the cyclic urethan carbonyl absorptions at 1760 and 1700 $cm.^{-1}$.

3-(2-Diethylaminoethyl)-2H-1,3-benzoxazine-2,4(3H)-dione hydrochloride (V) was prepared by the slow addition, with stirring, of 14.2 g. (0.13 mole) of ethyl chloroformate to 30.8 g. (0.13 mole) of *N*-(2-diethylaminoethyl)salicylamide in 200 ml. of xylene over a period of 10 min. After the addition was completed, the reaction mixture was heated to reflux with stirring for 1 hr. The mixture was cooled, and the resulting white hydrochloride was recrystallized from methanol–ether in a yield of 33.1 g.; the IR spectrum showed the cyclic urethan carbonyl absorptions at 1760 and 1700 $cm.^{-1}$. Compound V-2-^{14}C was prepared in a similar manner using ethyl chloroformate-^{14}C (carbonyl carbon).[3]

Compounds VI and VIII were synthesized in an analogous fashion.

Colorimetric Determinations—The addition of a 1% aqueous solution of ferric chloride[4] to unbuffered aqueous solutions of I resulted in the production of a violet color ($A_{max.}$ 538 mμ) which changed rapidly to a straw color ($A_{max.}$ 480 mμ). Despite the visible color change, the absorbance of such a solution at 520 mμ was found to be stable during the period of color change. Initial attempts to carry out this reaction in pH 7.4 buffered aqueous solutions of I were unsuccessful, as a white precipitate formed in phosphate buffer, a gelatinous orange precipitate formed in bicarbonate buffer, and a high blank value was observed with glycine buffer. Subsequently, 0.1 *N* borate buffer proved to be a suitable vehicle for the reaction. While the initial violet color, which changed rapidly to a straw color, was observed, the absorbance of the solution at 564 mμ, while not unchanging, was sufficiently stable to permit quantitation of the phenolic compounds.

The molar absorptivity of the ferric chloride–phenol complex was determined for each of the four compounds. Three solutions were prepared, each containing 1 mg. of the phenol/ml. of 0.1 *N* pH 7.4 borate buffer. Three 0.3-ml. aliquots of each solution were transferred into test tubes containing 2.7 ml. of the buffer. To each of the resulting solutions, 0.5 ml. of 1.0% aqueous ferric chloride was added, the mixtures were agitated briefly and the absorbance read at 564 mμ within 1.25 to 1.75 min. The molar absorptivity of each compound at 564 mμ was calculated.

Subsequently, three 10-mg. samples of the cyclic compounds were transferred into dry test tubes. At various zero times the aliquots were dissolved in 10 ml. of 0.1 *N* pH 7.4 borate buffer at room temperature (24°; a preliminary experiment revealed that the conversion of V to I occurred too rapidly for accurate study at 37°), and at

[2] A study of the amide hydrolysis of this compound has recently been reported (3).

[3] New England Nuclear.

[4] Centrifuged prior to use.

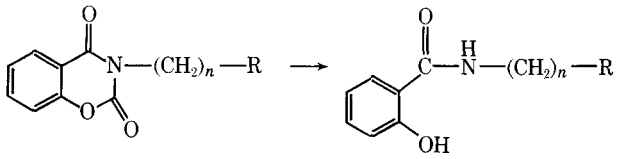


Table II—Rate of Conversion of the Cyclic Salicylamides to Their Phenolic Analogs in 0.1 *N* pH 7.4 Borate Buffer

Conversion[a]	*n*	R	Slope ± S[b]	r	Half-life of the Cyclic Compound, min.
V → I	2	$N(C_2H_5)_2$	−0.04910 ± 0.004	−0.96004	6.13[b]
VI → II	3	$N(C_2H_5)_2$	−0.08996 ± 0.003	−0.99536	200.40[c]
VII → III	2		−0.01635 ± 0.0004	−0.99419	18.41[d]
VIII → IV	3		−0.06508 ± 0.0008	−0.99763	277.20[e]

[a] At room temperature (24°). [b] or [d] *versus* [c] or [e], significant at the 0.001 level; [b] *versus* [d] and [c] *versus* [e] significant at the 0.001 level.

Table III—Influence of pH on the Conversion of Compound V to Compound I

System	pH	Relative Half-Life
0.1 *N* borate buffer	7.4	1.0
0.1 *N* phosphate buffer	7.0	1.1
0.1 *N* phosphate buffer	6.0	4.9
0.1 *N* acetate buffer	4.0	164.2
Artificial gastric juice	1.0	575.8

appropriate intervals 0.3-ml. aliquots of the solutions were transferred into test tubes containing 2.7 ml. of the buffer, 0.5 ml. of 1.0% aqueous ferric chloride was added immediately, the mixtures were agitated briefly and read at 564 mμ, against a buffer blank, within 1.25 to 1.75 min. The molar concentration of phenolic analog appearing in solutions of the cyclic compound was determined, and percent conversion, based on the maximum possible content of phenolic analog, was calculated.

Radiometric Determination—Since the ferric chloride assay proved to be unsuitable in buffers other than 0.1 *N* borate, which did not have the pH range required, V-2-^{14}C was employed in an investigation of the pH dependent nature of the conversion. In this study, the stability of the compound was investigated by collecting and measuring the carbon dioxide-^{14}C liberated during ring opening.

A solution was prepared containing 3.0 mg. V-2-^{14}C [approximately 70,000 (disintegrations per minute)]/50 μl. of freshly distilled propylene glycol. Three 50-μl. aliquots of this solution were transferred into carbon dioxide absorption tubes [described by Leeling and Phillips (8)], 3 ml. of buffer was added to each, and the assembled tubes were shaken at 88 excursions per minute on a platform shaker[5] at room temperature. A 1.5 × 3.8-cm. (0.6 × 1.5-in.) strip of filter paper[6] previously wet with 80 μl. of 0.5 *M* aqueous sodium hydroxide was suspended in the center of each tube to absorb the evolved carbon dioxide.

After shaking for varying periods of time, 0.2 ml. of 10 *M* aqueous sodium hydroxide was added to each tube through the side arm to prevent further evolution of carbon dioxide, and the shaking was continued for an additional 10 min. At the end of this time, the strips were removed, air-dried, and placed in Wheaton vials containing 17 ml. of a scintillator solution (80 g. of naphthalene, 10 g. of 2,5-diphenyloxazole, and 0.5 g. of 1,4-di[2-(5-phenyloxazolyl)]-benzene in a liter of a 1:3:3 mixture of xylene, dioxane, and ethylene glycol monoethyl ether). The resulting samples were counted in a liquid scintillation spectrometer[7] for a period of time sufficient to yield less than 2% counting error.

The amount of carbon dioxide-^{14}C liberated by complete conversion of V-2-^{14}C to I was determined by shaking the material in 0.1 *N* pH 7.4 borate buffer for 8 hr. In subsequent experiments, the amount of unchanged V-2-^{14}C was determined by relating all sample count rates to this value; this was necessary because the results of timed experiments do not provide absolute data regarding carbon dioxide-^{14}C production since some portion of the liberated gas is not trapped.

Statistics—Results, expressed as log percent cyclic salicylamide remaining unchanged with time, were subjected to regression analysis using the method of least squares (9). Half-lives were calculated using first-order rate equations discussed by Nelson (10). The significance of differences between appropriate slopes was tested by the Student's *t* test.

RESULTS AND DISCUSSION

Results obtained in the study of the conversion of four cyclic salicylamides to their phenolic analogs are summarized in Table II. These findings indicate that increasing the alkyl chain length from two to three carbons increases the stability of the cyclic compounds remarkably. Interestingly, the results also indicate that compounds containing the morpholine ring as the basic group are slightly, but significantly, more stable than the corresponding diethylamine analogs.

Results obtained in the study of the pH dependent nature of the conversion of V to I are summarized in Table III. These findings indicate clearly that the conversion is a base catalyzed pH dependent reaction. The results suggest that a significant portion of an oral dose of even an unstable cyclic salicylamide would remain unchanged prior to absorption.

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[5] Eberbach Corp. reciprocating shaker.
[6] Whatman No. 1.
[7] Packard Tri-Carb, model 314-EX.