TABLE III

ALKAMINE COMPOUNDS OF THE TYPE RS-C$_6$H$_3$(NH$_2$)-CR'=O

| R— | —R' | B. p., °C. | Hydrochloride M. p., °C. | mole HCl / mole base | N Analyses, % Calcd. | Found | Remarks |
|---|---|---|---|---|---|---|---|
| $CH_3$— | —$OCH_2CH_2N(C_2H_5)_2$ | 218 at 5 mm. | 168–172 | 1 | 8.79 | 8.71 | ..... |
| $C_2H_5$— | —$OCH_2CH_2N(C_2H_5)_2$ | 218–223 at 4 mm. | 118–123 | 1 | 8.43 | 8.38 | ..... |
| $n$-$C_3H_7$— | —$OCH_2CH_2N(C_2H_5)_2$ | 230–234 at 4 mm. | 190–194 | 1 | 8.09 | 8.21 | ..... |
| $CH_3$— | —$OCH_2CH_2CH_2N(C_2H_5)_2$ | 215–218 at 4 mm. | 175–179 | 1 | 8.43 | 8.24 | Hygroscopic |
| $C_2H_5$— | —$OCH_2CH_2CH_2N(C_2H_5)_2$ | 230–235 at 4 mm. | 166–171 | 1 | 8.09 | 8.04 | Hygroscopic |
| $CH_3$— | —$NHCH_2CH_2N(C_2H_5)_2$ | ..... | 196–200 | 2 | 11.86 | 11.93 | Hygroscopic |
| $C_2H_5$— | —$NHCH_2CH_2N(C_2H_5)_2$ | ..... | 149–151 | 2 | 11.44[a] | 11.36 | Hygroscopic |
| $n$-$C_3H_7$— | —$NHCH_2CH_2N(C_2H_5)_2$ | ..... | 145–149 | 2 | 11.02 | 11.09 | Hygroscopic |
| $C_6H_5CH_2$— | —$NHCH_2CH_2N(C_2H_5)_2$ | ..... | 197–201 | 2 | 9.77 | 9.72 | Hygroscopic |

[a] *Anal.* Calcd.: Cl, 19.07. Found: Cl, 18.80.

removed by distillation under diminished pressure, and the residual oil was dissolved in dry ether. Three molecular proportions of dry hydrogen chloride dissolved in ether was added dropwise with stirring, and the reaction mixture was allowed to stand in the refrigerator for several days in order to harden the precipitate. The dihydrochloride was dissolved in absolute alcohol, and dry ether which contained dry hydrogen chloride was added until the solution became faintly cloudy. The dihydrochloride crystallized on cooling, and was filtered from the solution, triturated with dry ether, and transferred to an Abderhalden drier. The yield was 3.25 g.

The properties of the alkamine esters and amides prepared and of their hydrochlorides are given in Table III.

All nitrogen analyses were run by the semi-micro Kejldahl method, carbon and hydrogen by semi-micro combustions, and sulfur by the Parr-bomb method. The analysis for ionizable chlorine reported in the footnote to Table III was run gravimetrically.

## Summary

1. Methods for the preparation of 3-nitro-4-alkylmercaptobenzoic acids and their simple esters, amides and acid chlorides have been developed. Examples of these compounds with four different alkylmercapto groups have been described.

2. The methyl esters and the amides of these acids have been reduced catalytically to the corresponding 3-amino-4-alkylmercaptobenzoic acid derivatives.

3. Five alkamine esters and four alkamine amides of the 3-amino-4-alkylmercaptobenzoic acids have been prepared from the methyl esters or amides and have been converted to their hydrochlorides for characterization.

NEW HAVEN, CONNECTICUT RECEIVED MARCH 4, 1947

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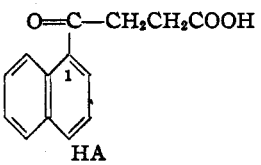
[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE OHIO STATE UNIVERSITY]

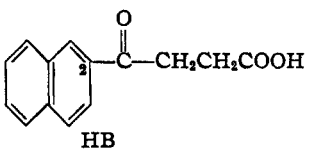
# Separation of Isomers. β-(2- and β-(1-Naphthoyl)-propionic Acid; their Ionization Constants and Solubilities and Method of Separation[1]

BY M. S. NEWMAN, ROBERT B. TAYLOR, THOMAS HODGSON AND A. B. GARRETT

The mixture of isomeric naphthoylpropionic acids



HA
β-(1-Naphthoyl)-propionic Acid



HB
β-(2-Naphthoyl)-propionic Acid

formed by the Friedel–Crafts condensation of succinic anhydride with naphthalene, is difficult to separate. We have found that if a solution of the sodium salts of these two acids is treated with mineral acid insufficient to liberate all of the acids, almost pure β-(2-naphthoyl)-propionic acid (hereinafter called HB, its isomer, HA) is precipitated. Successive additions of acid precipitate further amounts of HB until a point is reached after which further additions of acid precipitate HA and HB in nearly constant ratio.

This behavior is similar to that involved in the Mohr titration of chloride ion using the chromate ion as the indicator in which the chloride ion precipitates until the ratio of $[Cl^-]^2/[CrO_4^=] = 5 \times 10^{-9}$ at which point the two precipitate together.

However, in the case of these organic acids the values of the ionization constants are necessary, as well as the values of the solubilities, to determine the ionic precipitation ratio which we shall call $K$. This ratio, $K$, can be evaluated from the ionization constants $K_A$ and $K_B$ from HA and HB giving

$$\frac{[A^-]}{[B^-]} = \frac{K_A}{K_B} \times \frac{[HA]}{[HB]} = K \qquad (1)$$

(1) The material herein presented is taken in part from the M.S. Theses of R. B. T. and T. H.

If the degree of dissociation of the two acids is approximately the same then equation (1) becomes

$$\frac{[HA]}{[HB]} = K\frac{K_B}{K_A} = \text{ratio of the solubilities} \quad (2)$$

The value for this ionic precipitation ratio, $K$, as determined by substituting the experimental values, Table I, for $K_A$, $K_B$, [HA] and [HB] is about 7. The quantities [HA] and [HB] were

TABLE I

THE VALUES OF THE SOLUBILITIES AND IONIZATION CONSTANTS OF TWO ISOMERIC ACIDS AT 25°

| | Solubility in moles per 1000 g. of $H_2O$ | Ionization constant: From $pH$ and solubility of saturated solution | Ionization constant: At point where $pH = pK_a$ |
|---|---|---|---|
| | β-(1-Naphthoyl)-propionic Acid | | |
| S† | $3.5 \times 10^{-4}$ | $1.4 \times 10^{-5}$ | $2.1 \times 10^{-5}$ |
| U | 3.3 | 2.5 | 3.2 |
| S | 3.2 | 2.5 | 2.3 |
| S | 3.3 | 5.9 | 3.6 |
| U | 3.2 | 4.6 | 3.3 |
| Av. | $3.3 \times 10^{-4}$ | $3.4 \times 10^{-5}$ | $3.1 \times 10^{-5}$ |
| | | $3.3 \times 10^{-5}$ Av. | |
| | β-(2-Naphthoyl)-propionic Acid | | |
| U | $1.8 \times 10^{-4}$ | ..... | ..... |
| S | $1.0 \times 10^{-4}$ | ..... | ..... |
| U | $1.2 \times 10^{-4}$ | $1.13 \times 10^{-5}$ | $1.1 \times 10^{-5}$ |
| S | $1.1 \times 10^{-4}$ | $0.96 \times 10^{-5}$ | $1.1 \times 10^{-5}$ |
| U | $1.4 \times 10^{-4}$ | $.90 \times 10^{-5}$ | $1.3 \times 10^{-5}$ |
| S | $1.7^a \times 10^{-4}$ | $.79^a \times 10^{-5}$ | $1.3 \times 10^{-5}$ |
| Av. | $1.4 \times 10^{-4}$ | $.96 \times 10^{-5}$ | $1.2 \times 10^{-5}$ |
| | | $1.1 \times 10^{-5}$ Av. | |

[a] One determination only. † S = from side of supersaturation; U = from side of undersaturation.

calculated from the solubilities of the two acids corrected for their degree of dissociation (about 20%). The value of 7 indicates that there should be about 70% of HA and 30% of HB remaining in solution when the conditions are reached for constant ratio precipitation as outlined above.

This calculation was tested by the following experiment: A synthetic mixture of the sodium salts from 2.00 g. of HB and 1.00 g. of HA was fractionally acidified, giving 1.69 g. of fairly pure HB from the first fractions to be precipitated, whereas about 1.59 g. should have been recovered according to theory. For an experiment of this type this result is fairly satisfactory (see Table II).

TABLE II

COMPARISON OF THEORETICAL AND EXPERIMENTAL YIELDS IN THE SEPARATION OF THE ISOMERIC ACIDS FROM A SOLUTION CONTAINING 2.00 G. OF HB AND 100 G. OF HA DISSOLVED IN 13.40 ML. OF 1 $N$ SODIUM HYDROXIDE

| | Added 1 $N$ HCl, ml. | Acid pptd., g. | Calcd., g. | M. p., °C. | |
|---|---|---|---|---|---|
| 1 | 5.2 | 1.12 | 1.18 | 170.6–172 | Reasonably pure HB |
| 2 | 0.15 | 0.03 | 0.04 | 170.6–172 | |
| 3 | 1.5 | .34 | .36 | 170 –173 | |
| 4 | 1.0 | .20 | .23 | 172 –174 | |
| 5 | 1.5 | .35 | .36 | 121 –128 | |
| 6 | 4.0 | .92 | .83 | 121 –130 | |

[a] 2.96 g. recovered, 1% loss.

This method of partial acidification of a solution containing the dissolved salts of acids represents an additional method for the separation of isomeric or closely related acids. Used alone it is not as efficient as when used in conjunction with fractional crystallization of the various fractions. Specifically, the HB thus precipitated is not absolutely pure but by one crystallization from acetic acid reasonably pure HB can be obtained. The HA and HB remaining in solution will consist mostly of HA (the larger $K$, the greater the proportion of HA) so that recrystallization of the acid mixture obtained on further acidification yields pure HA. The method should work best when the more insoluble acid is also the weaker, as in the present instance.

Separation could also be attained by leaching mixtures of HA and HB with successive portions of alkali.

## Experimental

**β-(1-Naphthoyl)-propionic Acid.**—1-Bromonaphthalene boiling constantly at 149° at 20 mm. was prepared by fractional distillation. A solution of the Grignard reagent prepared from 34 g. of this bromide, 200 cc. of ether, 30 cc. of benzene and an excess of magnesium was added all at once to a stirred suspension of 17 g. of succinic anhydride in 100 cc. of 1:1 ether–benzene. After refluxing the mixture for several hours, the yellow complex formed was treated with dilute sulfuric acid and the keto acid produced was extracted with potassium carbonate solution. The crude colored acid obtained on acidification weighed 15.5 g. and melted at 122–127°. It was purified by conversion to the methyl ester, which was distilled (b. p. 196° at 3 mm.) and saponified. After three crystallizations from toluene, the colorless acid melted at 131.0–132.5°.

**β-(2-Naphthoyl)-propionic Acid.**—A sample of methyl 2-naphthyl ketone was purified by crystallization and distillation until it showed a long flat at 51.4° in a time-temperature cooling curve. This was converted into β-(2-naphthoyl)-propionic acid by the sequence of reactions used by Krollpfeiffer and Schafer.[2] The crude acid was purified as in the case of the 1-isomer. It formed a nicely crystalline product which had a sharp melting point at 173–174° but a slight purplish tinge in spite of repeated recrystallization using decolorizing carbon.

**Solubility Determinations.**—The equilibration procedure was the same as that of previous work.[3] The concentration of the acid in each solubility sample was determined by glass electrode titration with standard acid and with proper correction for hydrolysis.

### The Data

The data are summarized in Table I. The solubility values were obtained by titrating a known quantity of the saturated solution with 0.025 $N$ sodium hydroxide by means of a Coleman glass electrode. The glass electrode was calibrated against a standard buffer at $pH$ 4.1. The values of the ionization constants were determined by measuring the $pH$ value of the saturated solutions of the acid and also by determining the $pH$ at that point of neutralizations of the acid where the ratio $[HA]/[A^-]$ is unity. This is at the point of half-neutralization for acids that have a very low degree of dissociation; but these acids show approximately 20% ionization, hence the $pH^+ = pK_a$ at a point somewhat less than half-neutralization. This value can be obtained by the method of suc-

(2) Krollpfeiffer and Schafer, *Ber.*, **56**, 620 (1923).

(3) Johnston, Cuta and Garrett, THIS JOURNAL, **55**, 2311 (1933).

cessive approximations. By this method we first assume $[HA]/[A^-] = 1$ and determine the approximate degree of dissociation, this gives then a better value of the ratio $[HA]/[A^-]$ which can then be used to correct the ionization constant to a better value. The true value was obtained by repetitions of this procedure, a standard method for the solution of such problems.

All solubility and ionization constant data were obtained with the solutions under an atmosphere of nitrogen and with distilled water.

The maximum error in the average values of the solubilities is about $0.1 \times 10^{-4}$; in the values of the ionization constants (final average) $0.2 \times 10^{-4}$; and in the value of $K = [A^-]/[B^-]$ about 0.5.

**Separation of the Isomers.**—In a test case 2.00 g. of HB and 1.00 g. of HA were dissolved in 13.40 ml. of 1 *N* sodium hydroxide (calcd. 13.20 ml.) and varying amounts of 1 *N* hydrochloric acid were added. Each addition of acid was made with stirring while the solution was at 80°. After fifteen minutes the solution was cooled slowly during one hour to 25° and the acid was collected, weighed and dried. Melting points were taken on these fractions without recrystallization. The data are summarized in Table II.

## Summary

1. A method of separation of isomeric acids by utilizing the differences in their solubilities and ionization constants is described. The method is applied satisfactorily to the separation of β-(2-naphthoyl)-propionic acid and β-(1-naphthoyl)-propionic acid.

2. Values are given for the solubilities, the ionization constants and the per cent. ionization of these acids in saturated solution at 25°.

COLUMBUS, OHIO RECEIVED MAY 28, 1946

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF PARKE, DAVIS & CO.]

# Oxidative Degradation of Vitamin Bc (Pteroylglutamic Acid[1])

BY E. L. WITTLE, B. L. O'DELL, J. M. VANDENBELT AND J. J. PFIFFNER

Of the various methods we investigated as possible degradation routes for determination of the structure of vitamin Bc, oxidation using either neutral permanganate or a sodium chlorate–hydrochloric acid mixture appeared most promising when applied to small amounts of material and was most extensively used. While Angier, *et al.*, have disclosed the structure N-[4-{[(2-amino-4-hydroxy-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid (VI) and described a process for the synthesis of this compound, the degradative approach which we used differs from those which they have reported and we feel it is of interest to publish our findings. Oxidation of vitamin Bc gives rise to two main fragments, a pterine and a non-pterine part.

## The Pterine Part

Oxidation of vitamin Bc or its dimethyl ester with either permanganate or chloric acid following the procedure of Schöpf and Kottler[2] gave a 35% yield of a yellow, strongly fluorescent microcrystalline solid characterized by its chemical and physical properties as a pterine.[3] It contains a carboxyl group as shown by the formation of a methyl ester and was tentatively labeled acid 1. Because of its solubility properties the compound was very difficult to purify, especially in small quantity. The analytical results on this compound, its methyl ester and hydrochloride are listed chronologically in Table I and the ultraviolet absorption curves are graphed in Fig. 1 A.

As can be seen from Table I it was difficult to determine the formula for acid 1 from the analytical results, with the exception of the last two determinations 15 and 16 which fit excellently the formula $C_7H_5O_3N_5$ now known to be correct. While the first analytical results indicated a formula $C_8H_7O_3N_5$ for the acid, later analytical results, including a Van Slyke amino nitrogen determination and a halogen determination on the crystalline hydrochloride could only be fitted to the formula $C_9H_6O_4N_6$. The analytical values on the vitamin[4] itself when used with the non-pterine fragment ($C_{12}H_{12}O_5N_2Cl_2$) were of no help in deciding the formula of acid 1 since some of the analytical data fit equally well the formula $C_{19}H_{19}O_6N_7$ or $C_{21}H_{20}O_6N_8$ and thus left an $N_5$ or $N_6$ formulation possible for acid 1.

With the quantities of acid 1 available, degradation to simpler compounds of known structure was found to be difficult and largely unproductive. The only positive result in this direction was the oxidation with chloric acid from which guanidine, isolated and identified by means of its picrate, was obtained. The formation of this substance pointed to an unsubstituted 2-aminopyrimidine ring and strengthened the view that acid 1 was pterine in nature.

With both the analytical and degradative routes proving difficult, structure determination turned to the synthesis of probable pterine compounds. From a consideration of ultraviolet absorption curves it was possible to rule out pterines having a tautomeric oxygen atom at position 6 or 7 such

(1) Angier, *et al.*, *Science*, **103**, 667 (1946). A comparison of crystalline vitamin Bc isolated from liver and yeast with a synthetic sample of liver *L. casei* factor generously supplied by the Lederle Laboratories has demonstrated the identity of the compounds. When we reported the first successful isolation of a pure crystalline chick antianemia factor from liver, (*ibid.*, **97**, 404 (1943)) the designation vitamin Bc suggested by Hogan and collaborators for the chick antianemia factor was retained for the pure natural compound pending elucidation of its structure. Angier, *et al.* have achieved a chemical synthesis and suggested a suitable chemical name, pteroylglutamic acid, for this substance.

(2) Schöpf and Kottler, *Ann.*, **539**, 128 (1939).

(3) Schöpf, *Naturwissenschaften*, **30**, 368 (1942).

(4) THIS JOURNAL, **69**, 1476 (1947).