

SALTS OF SHIKIMIC ACID AND DERIVATIVES.*

II. SALTS OF AMMONIUM AND SUBSTITUTED AMMONIAS.**

BY HSING-HAN LEI.

If, as previously stated, the isolation of crystalline salts, notably of the alkali metals, may possibly be interfered with by the presence of the three hydroxy groups in shikimic acid, ammonium hydroxide apparently does not cause such interference. Results with other nitrogen basis were not always as favorable.

Ammonium Shikimate.—This was first prepared by Eykman¹ in 1891 and described by him. It was also prepared by S. Y. Chen² in 1929. Repetition of these experiments yielded satisfactory results.

Analysis: (By Kjeldahl method.)

1. 0.2125 Gm. of substance required 11.09 cc. of 0.1N HCl = 7.23 p. c. of N.
2. 0.3055 Gm. of substance required 11.22 cc. of 0.1444N HCl = 7.36 p. c. of N.

The theoretical percentage of nitrogen of the formula $C_6H_6(OH)_3COONH_4$ is 7.33 p. c.

Methylamine Shikimate.—This was prepared by S. Y. Chen in 1929,³ m. p. given was 162°. Repetition of his experiment yielded a compound with m. p. 163–164°.

n-Propylamine Shikimate.—This was prepared as follows: An excess of alcoholic propylamine solution was refluxed on a water-bath with alcoholic shikimic acid for about two hours. After the removal of most of the solvent by evaporation a thick sticky syrup was obtained which could not be crystallized.

n-Amylamine Shikimate.—This was prepared like the propylamine salt and yielded unsatisfactory results.

Benzylamine Shikimate.—This was made from a hot alcoholic mixture of shikimic acid and benzylamine. The reaction took place immediately. It crystallized in white needles from alcohol. M. p. 195–196°.

Nitrogen determinations according to the Kjeldahl method gave the following results:

1. 0.2825 Gm. of substance required 7.25 cc. of 0.1444N HCl = 5.19 p. c. N.
 2. 0.3352 Gm. of substance required 8.3 cc. of 0.1444N HCl = 5.01 p. c. N.
- | | |
|--|------------|
| Calculated for $C_6H_6(OH)_3COO.HN_3.CH_2C_6H_5$ | 4.98 p. c. |
| $C_6H_6(OH)_3CO.NH.CH_2C_6H_5$ | 5.33 p. c. |

Ephedrine Shikimate.—Equivalent amounts of shikimic acid and ephedrine were refluxed on a water-bath with 95 p. c. alcohol as solvent for about half an hour. Upon cooling the shikimate crystallized out as large crystals. They can be purified by recrystallizing from alcohol. M. p. 162–163°.

* Continued from JOUR. A. PH. A., 26, 902 (1937).

** From the laboratory of Edward Kremers.

¹ Ber., 24, page 1281. For a crystallographic study of the large crystals obtained from water see Groth, *Chemische Kristallographie*, 3, page 635.

² Am. J. Pharm., 101, page 689.

³ Ibid., 101, page 691.

Analysis: The determination of nitrogen was carried out by the Dumas method:

1. 0.2093 Gm. gave 8.5 cc. of N at 24° and 742.4 mm. = 4.46 p. c. N.
2. 0.1040 Gm. gave 4.3 cc. of N at 25° and 738 mm. = 4.49 p. c. N.

Calculated as addition product $C_{10}H_{15}ON.C_6H_6(OH)_3COOH$, N = 4.13 p. c.

Calculated as condensation product $C_{10}H_{15}ON.C_6H_6(OH)_3COOH$, N = 4.53 p. c.

Because elementary analysis did not seem satisfactory on account of the large molecule of ephedrine shikimate, the salt was resolved into its components. The ephedrine was determined by the U. S. P. XI method (page 146) for the assay of ephedrine sulfate, the shikimic acid gravimetrically as its ammonium salt.

0.5-Gm. sample yields 0.24286 Gm. of ephedrine (10.3 cc. of 0.09972N NaOH was used for titrating back of 20 cc. 0.1249N HCl or 14.71 cc. of *N*/10 HCl used for titrating the base liberated) and 0.2811 Gm. of ammonium shikimate, corresponding to 48.57 p. c. of ephedrine and 51.22 p. c. of shikimic acid, respectively.

Calculated for $C_{10}H_{15}ON.C_6H_6(OH)_3COOH$: Base = 48.67 p. c.; acid = 51.33 p. c.

Aniline Shikimate.—This was reported by S. Y. Chen in 1929¹ melting point given as 178°. Repetition of the experiment yielded a powder m. p. 194–195°. In aqueous solution it is acid to litmus paper.

Analysis gave the following results:

1. 0.245 Gm. of substance required 9.3 cc. of 0.1N HCl = 5.32 p. c. N.
2. 0.252 of substance required 9.6 cc. of 0.1N HCl = 5.33 p. c. N.

Calculated for $C_6H_5(OH)_3COO.NH_3.C_6H_5$ 5.25 p. c.

$C_6H_5(OH)_3CONH.C_6H_5$ 5.62 p. c.

o-Toluidine Shikimate.—This was made by refluxing the alcoholic mixture of shikimic acid and base on a water-bath for one hour. The product was a powder. M. p. 178–180°.

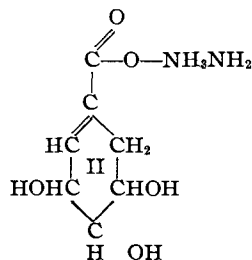
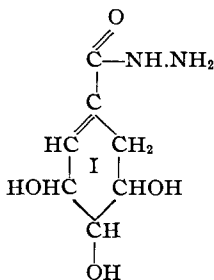
Analysis gave the following results:

1. 0.2035 of substance required 7.3 cc. of 0.1N HCl = 5.03 p. c. N.
2. 0.2145 of substance required 7.8 cc. of 0.1N HCl = 5.09 p. c. N.

Calculated for $C_6H_5(OH)_3COO.NH_3.C_6H_4.CH_3$ 4.98 p. c.

$C_6H_5(OH)_3CONH.C_6H_4.CH_3$ 5.33 p. c.

Hydrazine Shikimate.—In an attempt to produce a condensation product of hydrazine (I) with shikimic acid, an addition product (II) resulted.



¹ *Am. J. Pharm.*, 101, page 691.

When equivalent amounts of the two substances were mixed at room temperature a thick paste resulted with the evolution of appreciable heat. After heating for ten minutes on a water-bath, alcohol was added and the heat continued for an hour, supplemented by a mechanical stirrer. A white crystalline product resulted, m. p. 147–148°.

The nitrogen content was determined by the Dumas method.

1. 0.1045 Gm. yielded 13.1 cc. N at 30° and under 733-mm. pressure, corresponding to 13.14 p. c. N.
2. 0.1068 Gm. yielded 13.2 cc. N at 30° and under 743-mm. pressure, corresponding to 13.13 p. c. N.

	I.	II.
Computed for	14.89 p. c.	13.59 p. c.

Pyridine Shikimate.—This was prepared by S. Y. Chen in 1929,¹ m. p. 184°. Repetition of the experiment yielded microscopic plates m. p. 184–185°.

Analysis gave the following results:

1. 0.291 Gm. of substance required 11.01 cc. of 0.1N HCl = 5.30 p. c. N.
2. 0.3012 Gm. of substance required 11.8 cc. of 0.1N HCl = 5.49 p. c. N.

Calculated for $C_6H_5(OH)_3.COOHC_6H_5$ 5.54 p. c.

Quinine Shikimate.—This was prepared as follows: Two grams of shikimic acid were dissolved in 10 cc. of water with the aid of heat. An excess of quinine base was then added, shaking until most of the base had gone into solution. The solution was filtered upon cooling. Quinine shikimate crystallized out as colorless needles. The salt can be crystallized from alcohol, 221–222°.

Analysis: By modifying the methods described in Jenkins and DuMez, "Quantitative Pharmaceutical Chemistry," page 297, the base was determined gravimetrically.

1. 0.3666-Gm. sample yielded 0.2382 Gm. quinine corresponding to 64.97 p. c.
2. 0.3604-Gm. sample yielded 0.2335 Gm. quinine corresponding to 64.80 p. c.

The calculated amount for $C_6H_5(OH)_3COOH.C_{20}H_{24}O_2N_2$ is 65.08 p. c.

Quinidine Shikimate.—This salt was prepared like that of quinine. From diluted (60 p. c.) alcohol it was obtained in colorless needles, m. p. 224–226°.

When heated on an air-bath for four hours at a temperature of between 100° and 110° it did not lose in weight, indicating the absence of water of crystallization. The melting point of the salt thus treated was 225–226°.

Because of the large molecule, elementary analysis did not seem satisfactory. Hence it was resolved into its components. The quinidine was determined by the U. S. P. XI method (page 126) for the assay of codeine phosphate; the shikimic acid gravimetrically as its ammonium salt.

0.56 Gm. yielded 0.3650 Gm. of quinidine and 0.2162 Gm. of ammonium shikimate, corresponding to 65.18 p. c. of quinidine and 34.97 p. c. of shikimic acid, respectively.

Calculated for $C_{20}H_{24}O_2N_2.C_6H_5(OH)_3COOH$: Base = 65.08 p. c.; acid = 34.92 p. c.

Codeine Shikimate.—This salt was prepared like those of quinine and quinidine. From dilute alcohol it was obtained in crystals somewhat resembling cubes, m. p. 173–174° (sintered at 160°).

¹ *Am. J. Pharm.*, 101, page 691.

The analysis was carried out by the Dumas method:

1. 0.1015 Gm. gave 2.9 cc. N at 738.6 mm. and $27.5^\circ = 3.06$ p. c. N.
2. 0.1005 Gm. gave 2.9 cc. N at 738 mm. and $28^\circ = 3.04$ p. c. N.

Calculated for $C_{18}H_{21}O_3N.C_6H_6(OH)_3COOH$ 2.96 p. c.

Strychnine Shikimate.—This was prepared from the strychnine base and aqueous shikimic acid as carried out in the preparation of the quinine salt. The salt crystallized out as colorless long plates, m. p. $234-236^\circ$ (sintered at 154°).

Analysis: This was carried out as for quinine shikimate except that chloroform was used instead of ether as the extracting solvent.

1. 0.1075-Gm. sample yielded 0.078 Gm. strychnine corresponding to 72.5 p. c.

Calculated for $C_6H_6(OH)_3COOH.C_{21}H_{22}O_2N_2.2H_2O$ is 75.63 p. c.

REFERENCES.

- (1) Eykman, J. F., *Ber.*, 24, 1281 (1891).
- (2) Chen, S. Y., *Am. J. Pharm.*, 101, 689 (1929).
- (3) Chen, S. Y., *Ibid.*, page 691.
- (4) Chen, S. Y., *Ibid.*, page 691.
- (5) Chen, S. Y., *Ibid.*, page 691.

TOXICITY IN THE LEAVES OF RHODODENDRON CALIFORNICUM, HOOK. I.*

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Since the days of Xenophon, in the fifth century B. C., it has been known that certain rhododendrons were poisonous, but all systematic investigation (1) of this toxicity dates since 1885, during which period research has been carried out on 15 species, of which 13 were found to contain poison.

Rhododendron californicum, which abounds in the forests of northern California and of western Oregon, Washington and British Columbia, was not one of the 15 species investigated. Although various botanical handbooks (15) state that the plant is said to be poisonous, no reference was found in the literature to any research on that particular species. The following report represents a preliminary investigation on the toxicity of this rhododendron.

EXPERIMENTAL.

Materials.—Leaves were collected in June 1935, and also in December 1936, along the Oregon coast near Newport and near Florence. Both samples were immediately air-dried indoors, losing about 58% of their weight during the curing. The dried leaves still contained about 16.4% of moisture, corresponding to 64.9% in the green leaves. The ash content was about 2.9% on the cured leaves, corresponding to about 1.2% in the green state.

Extraction with Alcohol.—Three different concentrations of alcohol, 15%, 45% and 95% were employed in extracting the ground dried leaves. The yields were 37%, 44.5% and 35%, respectively, but from the dried extract so produced, no crystalline substance could be obtained, nor other material with marked physiological activity, wherefore the method was abandoned. It is

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¹ Abstracted from a thesis presented by Chieko Otsuki in partial fulfillment of the requirements for the degree of Master of Science from the Oregon State College.