Title	Dissociation constants of certain γ-pyrone dicarboxylic acids : meconic acid and chelidonic acid
Sub Title	
Author	宮本, 貞一(Miyamoto, Sadaichi) Brochmann-Hanssen, Einar
Publisher	共立薬科大学
Publication year	1962
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.6(1961)/7(1962) ,p.53- 58
JaLC DOI	
Abstract	
Notes	
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000006-0053

SUMMARY

Attempts have been made to determine the origin of opium on the basis of the content of meconic acid, sulfuric acid and total acid. Simple and rapid procedures have been developed for determination of these acids. The results show that the method is capable of differentiating between opium from several geographic regions. It may find application as a means of confirming the results obtained by another independent method.

REFERENCES

1. Lj. Grlic, U. N. Document ST/SOA/SER.K/ 54(1957).
2. United Nations Secretariat, Docnment ST/SOA/SER.K/65(1958).
3. A. Jabbar and E. Brochmann-Hanssen, U.N. Document ST/SOA/SER.K/102(1960).
4. A. F. Witte, U. N. Document ST/SOA/SER.K/64 (1958).
5. H. Asahina and M. Ono, U. N. Document ST/SOA/SER.K/90 (1959).
6. E. Brochmann-Hanssen, J. Am. Pharm. Assoc., Sci. Ed.,45, 74(1956).
7. E. Brochmann-Hanssen, Medd. Norsk Farm. Selskap, 17, 76(1955).
8. E. Krogh-Svendsen, J. Zimmermann and F. Reimers. Medd. Norsk Farm. Selskap, 18, 97, 113(1956).
9. J. Zimmermann and F. Reimers, Dansk Tiddsskr. Farm., 30, 77 (1956), and 31, 202 (1957)
10. J. Zimmermann, E. Krogh-Svendsen and F. Reimers, Analytica Chimica Acta, 16, 6 (1957).
11. "Three-Way Analysis with the Fisher Nefluoro-Photometer," Fisher Scientific Company, Pittsburgh, Pennsylvania, p. 55.

Dissociation Constants of Certain γ-Pyrone Dicarboxylic Acids*
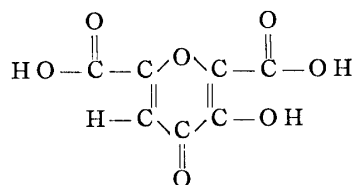
Meconic Acid and Chelidonic Acid

By Sadaichi MIYAMOTO and Einar Brochmann-Hanssen

Meconic acid and chelidonic acid are very strong acids having dissociation
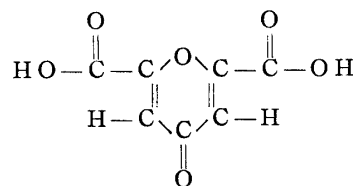constants of the same order of magnitude as sulfuric acid.
This high acidity is explained on the basis of
the resonance structure of the γ-pyrone molecule.

The two main acids of opium are meconic acid and sulfuric acid, which together account for 60 to 80% of the total acid content (1). In the course of our studies of the acid composition of opium it became apparent that meconic acid is an exceptionally strong acid, from the point of view of being an organic carboxylic acid. Search of the literature failed to reveal any information about the extent of its acid strength. This led to an investigation of the dissociation constants of meconic acid and the closely related chelidonic acid.

$$HO-\underset{\substack{\|\\O}}{C}-C\diagdown_{\substack{O\\\diagup}}C-\underset{\substack{\|\\O}}{C}-OH$$

$$H-C\diagdown_{\substack{C\\\diagup}}C-OH$$

Meconic acid

$$HO-\underset{\substack{\|\\O}}{C}-C\diagdown_{\substack{O\\\diagup}}C-\underset{\substack{\|\\O}}{C}-OH$$

$$H-C\diagdown_{\substack{C\\\diagup}}C-H$$

Chelidonic acid

EXPERIMENTAL

Preparation of Meconic Acid.—Ten grams of powdered opium was mixed with 50 Gm. of Dowex 50-X2 (H⁺), 50-100 mesh, and 500 ml. of hot water in a 1 L. Erlenmeyer flask. The mixture was shaken mechanically for 30 minutes. The alkaloids, amino acids, and most of the colored materials were adsorbed on the resin, while the organic and inorganic acids were liberated. The supernatant liquid was decanted, 250 ml. of hot water was added to the resin, and the shaking was continued for 15 minutes. This process was repeated. The cmbined acid extracts were concentrated to about 100 ml. under reduced pressure, and powdered barium hydroxide was added until the solution was distinctly basic to litmus. The precipitate which fomed was collected by centrifugation and washed several times by suspension in a little cold water, followed by centrifugation. It was treated repeatedly with small portions of 1 N hydrochloric acid to dissolve barium meconate, leaving a residue of barium sulfate. A test for complete removal of barium meconate was made by means of ferric chloride reagent (5% ferric chloride in 0.1 N hydrochloric acid). This reagent giver a red color with meconic acid. The combined extracts of barium meconate were neutralized with 10% ammonia, whereby barium meconate again precipitated out. The suspension was centrifuged, the precipitate washed several times with small amounts of cold water, and then transferred to a 250-ml. Erlenmeyer flask with the aid of about 100 ml. of water. Ten grams of Dowex 50-X₂(H⁺), 50-100 mesh, was added and the mixture was shaken mechanically for 30 minutes. The solution, containing free meconic acid, was filered through a pledget of glass wool and the ion exchange resin was washed with water. The combined filtrate and washings were concentrated under reduced pressure at a temperature below 50°. Meconic acid crystallized and was recrystallized several times from water as the trihydrate. Titration with 0.02 N sodium hydroxide gave a purity of 99.8%. Anhydrous meconic acid was obtained by heating to constant weight at 102°. Upon titration the anhydrous sample showed a purity of 100±0.2%.

Preparation of Chelidonic Acid.—Chelidonic acid was synthesized from ethyl oxalate and acetone according to the method described by Riegel and Zwilgmeyer (2). It was recystallized from water as the monohydrate with a purity of 99.5%. Anhydrous chelidonic acid was obtained by heating at 160° to constant weight.

Determination of Dissociation Constants.—*Titration Method.*—Solutions of meconic acid and chelidonic acid (10⁻³ M) were titrated potentiometrically at 25° using a Beckman model W pH meter in combination with a Brown recording potentiometer, the scale of which was graduated in pH units. The titrant, 1 N sodium hydroxide, was added at a slow, constannt rate by means of a synchronous clock motor driving the plunger of a small syringe. A polyethylene capillary tubing led from the syringe into the solution being titrated. The titration curves, which are illustrated

Table I.—Dissociation Constants for Meconic Acid and Chelidonic Acid Calculated from the Neutralization Curve at 25°

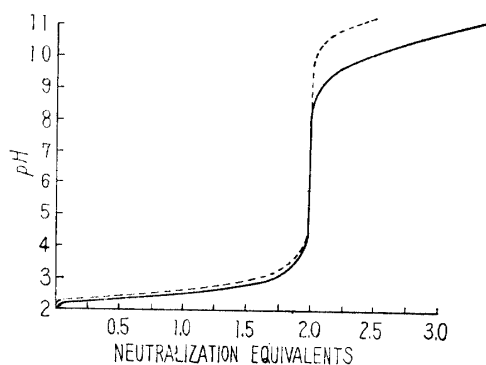| Neutralization Equivalents of Alkali	$(Na^+) \times 10^2$	pH		pK	
		Meconic Acid	Chelidonic Acid	Meconic Acid	Chelidonic Acid
0.25	1.25	2.23	2.32	......	......
0.50	2.50	2.30	2.38	......	......
0.75	3.75	2.39	2.47	......	......
1.25	6.25	2.62	2.70	2.20	2.43
1.50	7.50	2.79	2.88	2.12	2.37
1.75	8.75	3.09	3.17	2.07	2.28
2.25	11.25	9.68	......	10.16	......
2.50	12.50	10.09	......	10.09	......
2.75	13.75	10.44	......	10.05	.....

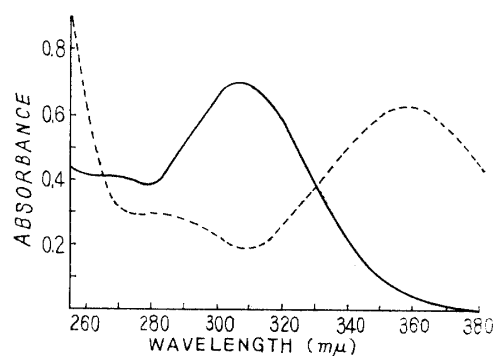Fig. 1.—Titration curves for meconic acid (solid line) and chelidonic acid (broken line).

Fig. 2.—Ultraviolet spectrum of meconic acid in 0.1 N hydrochloric acid (solid lino) and in 0.1 N sodium hydroxide (broken line).

Table II.—Buffer Solutions Used

pH Range	Composition	Ionic Strength
1.0-2.8	$HCl + KCl$	0.015
3.0-5.6	$CH_3COOH + CH_3COONa$	0.02
6.0-7.8	$KH_2PO_4 + NaOH$	0.02
8.0-11.47	$H_3BO_3 + NaOH$	0.03

in Fig. 1, show that both acids are very strong acids giving a sharp change in pH at the neutralization point. Chelidonic acid produces a single break after the addition of two equivalents of alkali, indicating that the two dissociation constants are relatively close. Meconic acid exhibits a second, but much less distinct potential change after addition of three epuivalents of base.

The dissociation constants were calculated from the following equation

$$K_a = \frac{[H^+]\left[(Na^+) + (H^+) - \frac{Kw}{(H^+)}\right]}{M - \left[(Na^+) + (H^+) - \frac{Kw}{(H^+)}\right]}$$

where M is the total molar concentration of the acid. Calculations were made at the 0.25, 0.5, and 0.75 neutralization points (Table I). No value could be obtained for pK_1, since the calculations yielded negative results for this constant.

Spectrophotometric Method.—The results obtained by the titration method were checked by a spectrophotometric procedure (3). Meconic acid and chelidonic acid were found to obey Beer's law in the range of 230 mμ in both acidic, neutral, and basic solutions. Buffer solution were prepared in range of pH 1 to 11.5. These were adjusted to constant ionic strength by means of sodium chloride and diluted with water to an ionic strength of 0.015 to 0.03 (Table II). Solutions of hydrochloric acid and sodium hydroxide in concentrations of 0.1 N and 1 N were also used for pH adjustment.

The determinations were carried out as follows: in a 25-ml. volumetric flask was placed 0.25 ml. of a slution of meconic acid or chelidonic acid, 5×10^{-3} M, and the volume was adjusted to the mark with buffer selution (or with hydrochloric acid or sodium hydroxide). Thus, a final concentration or 10^{-5} M was obtained. The final pH of the solution was determined with a Beckman model G pH meter and the absorbance was read in a Beckman DU spectrophotometer at 25°, The following wavelengths were chosen to give maximum change in absorbance with a change in pH; for meconic acid: 295, 305, and 315 mμ (Fig. 2); for chelidonic acid: 250, 260, and 270 mμ.

The degree of ionization (α) was calculated from the following equation

$$\alpha = (k_a - k_m) / (k_i - k_m)$$

where k_m = molar absorptivity of the unionized form, k_i = molar absorptivity of the completely ionized form, and k_a = molar absorptivity of the partly ionized form. Then, pK was calculated

Table III.—Determination of pK_3 of Meconic Acid by Spectrophotometry at 25°

pH	295 mμ A	295 mμ pK	305 mμ A	305 mμ pK	315 mμ A	315 mμ pK
7.17	0.411	⋯⋯	0.443	⋯⋯	0.366	⋯⋯
7.70	0.411	⋯⋯	0.443	⋯⋯	0.366	⋯⋯
7.99	0.409	10.133	0.442	9.528	0.364	10.109
8.27	0.407	10.103	0.438	10.097	0.363	10.206
8.65	0.401	10.074	0.432	10.125	0.357	10.096
8.94	0.395	10.148	0.423	10.142	0.351	10.154
9.08	0.386	10.079	0.412	10.076	0.342	10.073
9.53	0.352	10.092	0.372	10.098	0.309	10.082
9.91	0.295	10.044	0.305	10.071	0.256	10.045
10.19	0.246	10.010	0.247	10.050	0.212	10.028
10.50	0.200	9.978	0.185	9.992	0.172	10.032
12.48	0.142	10.750[a]	0.110	10.433	0.138	11.627[a]
14.00	⋯⋯	⋯⋯	⋯⋯	⋯⋯	⋯⋯	⋯⋯
(1 N NaOH)	0.137	⋯⋯	0.105	⋯⋯	0.106	⋯⋯

[a] Extreme values not included in calculation of mean.

as follows

$$pK = pH - \log [(\alpha)/(1-\alpha)]$$

In the region of low pH values there was very little change in absorbance with a change in pH. It was, therefore, not possible to differentiate clealy between the molecular form and the ionized form onthe basis of spectral changes for the first and second dissociation constants. Consequently, no dependable values could be obtained. The third dissociation constant for meconic acid was readily calculated and gave results that agreed closely with those obtained by the titration method (Table Ⅲ).

Determination of Dipole Moment.—The dipole moment of anhydrous meconic acid was measured in dioxane at 30° (4). No measurement was made for chelidonic acid because it was not sufficiently soluble in dioxane or any other solvent suitable for dipole moment studies.

RESULTS AND DISCUSSION

The overall results are summarized in Table Ⅳ. The ionization constants reported are mean values based on Table I and Table Ⅲ.

The results show that both meconic acid and chelidonic acid are very strong acids having dissociation constants very close to those given for sulfuric acid (5). This high acidity is probably due to the peculiar character of the γ-pyrone structure favoring a separation of charge by participation of the benzenoid form in a resonance hybrid
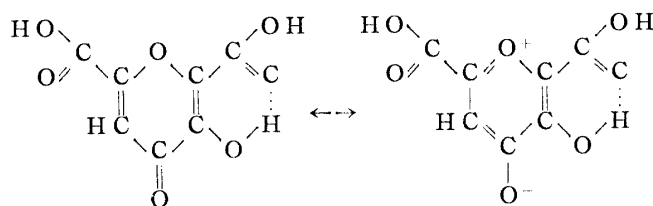
The positive charge on the ring oxygen will be expected to increase the acidity by the inductive effect. The high dipole moment for meconic acid also supports this hypothesis.

γ-Pyrone itself has a dipole moment of 4 D (6). It has been calculated that if γ-pyrone were completely in the form with a separation of charge, its dipole moment would be 22 D (6). Likewise, it has been estimated that its keto form would have a dipole moment of 1.75 D (7). The actual situation is, therefore, a resonance hybrid to which both forms contribute.

The inductive effect of the hydroxyl group and hydrogen bonding between the hydroxyl group and the carboxyl group would tend to increase the acid strength of the latter. This would explain why meconic acid is a stronger acid than chelidonic acid.

Infrared spectra indicated the presence of hydrogen bonding in meconic acid, but were otherwise not very helpful for characterization of the structure.

Opium contains about 7 to 13% meconic acid and 2 to 4% sulfuric acid (1). The presence of such high concentrations of very strong acids is probably biologically

Table IV.—Dissociation Conrtants for Meconic Acid and Chelidonic
Acid and the Dipole Moment of Meconic Acid

	Meconic Acid	Chelidonic Acid
pK_1	......	......
pK_2	2.23	2.36
pK_3	10.10^a (10.08^b)	......
μ	5.19 D	......

[a] Titration method. [b] Spectrophotometric method.

significant in view of the high alkaloidal content of opium. In the same connection, it is interesting to note that *Chelidonium majus* L., in which the alkaloids also are localized in the latex, contains chelidonic acid, another strong dibasic acid.

REFERENCES

(1) Miyamoto, S., and Brochmann-Hanssen, E., United Nations' Secretariat, Document ST/SOA/SER.K 106.

(2) Riegel, E. R., and Zwilgmeyer, F., in Blatt, A. H., "Organic Synthesis," Coll. Vol. 2, John Wiley & Sons, Inc. New York, N. Y., 1943, p. 126.

(3) Sager, E. E., Schooley, M. R., Carr, A. S., and Acree, S. F., *J. Research Natl. Bvr. Standards*, 35,521(1945)

(4) Halverstadt, I. F., and Kumler, W. D., *J. Am. Chem. Soc.*, **64**, 2988(1942).

(5) Lange, M. A., "Handbook of Chemistry," Handbook Publishers, Inc., Sadusky, Ohio, 1956, p. 1202.

(6) Albert, A., "Heterocyclic Chemistry," The Athlone Press, University of London, 1959, p. 270.

(7) Hunter, E. C. E, and Partington, J. R., *J. Chem. Soc.*, 1933, 87.

アンスロン試薬によるトリプトファンの呈色反応に関する研究 (第1報*)

中 村 勇 蔵

Studies on the Coloration of Tryptophan by Anthrone Reagent. I.

Yuzo NAKAMURA

アンスロン試薬による糖の定性ならびに定量分析については多数の報告があるが，著者は数種のアミノ酸の共存がこの反応に影響を与えることを知つたので，まずトリプトファンにつき，数種の糖の共存下に実験を行ない，その結果特異的な紫色を呈することを認めた．すでに Shetlar[1] は血清中の多糖類定量の目的にアンスロン試薬を用いた場合に 530 mμ に吸収極大を有する呈色を認め，これがトリプトファンの共存に基因するのであり，したがつてアンスロン試薬による血清や組織中の多糖類定量に際しては，この影響を考慮すべきであることを指摘し，また Tuller[2] 等はこの反応を利用したタンパク質含有物，すなわち糖タンパク体あるいはその他のタンパク体が共存する糖の定量法を発表している．しかし従来この反応の条件，共存する糖およびアミノ酸の種類などによる影響によつて生ずる差違，またこの際形成される色素の性質などに