(0.02 *M*, *p*H 7.0). The clear supernatant obtained by high speed centrifugation (25,000 *g*, 30 minutes) was treated with aluminum hydroxide Cγ gel. The enzyme was eluted with 0.2 *M* potassium phosphate buffer (*p*H 7.5) and the eluate was dialyzed against tris-hydroxymethylaminomethane buffer (0.01 *M*, *p*H 7.0) for 5 hours at 0°.

The stoichiometry of the reaction is shown in Table I. When corrected for the spontaneous decarboxylation of oxaloacetate, the disappearance of oxaloacetate[1] was matched by the appearance of almost equal amounts of oxalate and acetate. Under these conditions, none of the following compounds yielded oxalate: malate, fumarate, succinate, tartrate, oxalosuccinate,[2] citrate, *cis*-aconitate, isocitrate, pyruvate, glycolate, glyoxylate and acetate.

TABLE I

STOICHIOMETRY OF OXALOACETATE CLEAVAGE

Expt. I: a reaction mixture (2.0 ml.) containing 20 μmoles of oxaloacetate, 10 μmoles of $MnCl_2$, 200 μmoles of tris-hydroxymethylaminomethane buffer *p*H 8.1 and the enzyme (3.7 mg. protein) was incubated at 37° for 30 minutes. Expt. II: without $MnCl_2$. Expt. III: boiled enzyme control. Zero time samples did not contain any oxalate. Numbers are expressed in μmoles.

	Δ Oxaloacetate[a]	Δ Pyruvate[b]	Δ Acetate[c]	Δ Oxalate[d]
Expt. I	−17.3	5.6	11.6	11.1
Expt. II	−12.2	5.2	6.8	6.0
Expt. III	−6.7	7.0	0	0

[a] Determined by the method of A. H. Mehler, A. Kornberg, S. Grisolia and S. Ochoa, *J. Biol. Chem.*, **174**, 961 (1948). [b] Determined by the method of A. Kornberg and W. E. Pricer, Jr., *ibid.*, **184**, 769 (1950). [c] Acetate was determined with acetokinase from *E. coli:* I. A. Rose, M. Grunberg-Manago, S. R. Korey and S. Ochoa, *ibid.*, **211**, 737 (1954). We are indebted to Drs. E. Heath and J. Hurwitz for their help and a generous gift of a purified acetokinase preparation. The acetohydroxamate formed in this assay was further identified by paper chromatography according to E. R. Stadtman and H. A. Barker, *ibid.*, **184**, 769 (1950). [d] Oxalate was determined manometrically with a highly purified preparation of oxalic decarboxylase from *Collyvia veltipes* (H. Shimazono and O. Hayaishi, unpublished procedure).

In a large scale experiment oxalate was precipitated as the calcium salt, dissolved in 0.1 *N* HCl, passed through a Dowex-50 (H^+-form) column and crystallized as the free acid. Its identity was established by melting point, analysis and a comparison of the infrared spectrum with that of an authentic sample.

The enzyme required Mn^{++} for maximum activity but no other cofactors appeared to be involved. The hydrolytic cleavage of oxaloacetate to yield acetate and oxalate has been postulated by previous investigators.[3,4] Although further purification of the enzyme appears to be necessary to clarify the precise mechanism of the reaction, the results presented in this paper represent the first experimental evidence for this hypothetical reaction. This enzyme is therefore tentatively designated as oxaloacetic hydrolase.

(1) Samples of oxaloacetic acid (m.p. 150 ~ 152°) were purchased from California Foundation for Biochemical Research and Sigma Chemical Co. Oxaloacetic acid (m.p. 182 ~ 5°), presumably hydroxyfumaric acid, was obtained from Sigma Chemical Co. The three preparations gave essentially identical results.

(2) A commercial preparation of oxalosuccinic acid contained approximately 16% oxalic acid as an impurity.

(3) H. Raistrick and A. B. Clark, *Biochem. J.*, **13**, 329 (1919).

(4) F. Lynen and F. Lynen, *Ann.*, **560**, 149 (1948).

(5) Fellow of The Jane Coffin Childs Memorial Fund for Medical Research.

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RECEIVED AUGUST 2, 1956

THE VINYL SIDE-CHAIN IN GELSEMINE[1]

Sir:

Gelsemine contains a double bond that readily can be hydrogenated[2] and has been considered heretofore as being located in an exocyclic methylene group.[3,4] This assignment was based on the interpretation of two reactions.[3,4] Dr. E. Wenkert at a recent meeting of the American Chemical Society questioned the validity of the interpretation, thus prompting a further study of the nature of the double bond.

Cleavage of the olefinic bond in N(a)-methylgelsemine ($C_{21}H_{24}O_2N_2$) by sodium metaperiodate in the presence of a catalytic amount of osmium tetroxide[5] gave rise to a substance, m.p. 192–194°, (Calcd. for $C_{20}H_{22}O_3N_2$: C, 70.98; H, 6.55; N, 8.28; 2N-CH_3, 8.88; C-CH_3, nil. Found: C, 71.10; H, 6.62; N, 8.53; N-CH_3, 8.61; C-CH_3, nil.) The infrared absorption spectrum of this substance contained, besides a band at 1702 cm.$^{-1}$ due to the oxindole carbonyl, a second band in the same region (1719 cm.$^{-1}$) and one at 2735 cm.$^{-1}$ characteristic of the carbonyl and of the CH stretching respectively of an aldehyde. Reaction of the oxidation product with hydroxylamine gave an oxime, m.p. 267–268°, (calcd. for $C_{20}H_{23}O_3N_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 68.21; H, 6.75; N, 11.81) which, on dehydration with acetic anhydride and pyridine, produced a nitrile, m.p. 240–241°, (calcd. for $C_{20}H_{21}O_2N_3$: C, 71.62; H, 6.31; N, 12.53. Found: C, 71.57; H, 6.42; N, 12.49). The infrared absorption spectrum of this nitrile showed a band at 1711 cm.$^{-1}$ attributable to the oxindole carbonyl and a sharp band at 2255 cm.$^{-1}$ characteristic of the C≡N vibration. The formation of a nitrile under these conditions confirms that the oxidation product was indeed an aldehyde, and not a ketone. This was further confirmed by the Wolff–Kishner reduction of the oxidation product which gave rise to a compound, m.p. 172–174°, (calcd. for $C_{20}H_{24}O_2N_2$: C, 74.04; H, 7.46; N, 8.64; 1C-CH_3: 4.63. Found: C, 74.19; H, 7.56; N, 8.79; C-CH_3, 4.11). The infrared absorption spectrum of this compound contained the oxindole carbonyl band at 1707 cm.$^{-1}$, but no

(1) Issued as N.R.C. Bull. No. 4105.

(2) T. T. Chu and T. Q. Chou, THIS JOURNAL, **62**, 1955 (1940).

(3) M. S. Gibson and R. Robinson, *Chem. and Ind.*, 93 (1951).

(4) R. Goutarel, M. M. Janot, V. Prelog, R. P. A. Sneeden and W. I. Taylor, *Helv. Chim. Acta*, **34**, 1139 (1951).

(5) R. Pappo, D. S. Allen, Jr., R. U. Lemieux and W. S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).

longer contained the bands at 1719 and 2735 cm.$^{-1}$ attributable to the aldehyde function.

These results lead to the unequivocal conclusion that the olefinic double bond in gelsemine is present in a vinyl side chain, and not in an exocyclic methylene group as had heretofore been assumed.

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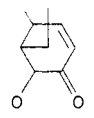
RECEIVED AUGUST 22, 1956

CODEINONE DIMETHYL KETAL AND ITS CONVERSION TO THEBAINE

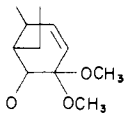
Sir:

Thebaine is prodigious among morphine alkaloids for the number and variety of its transformation products.[1] However, no morphine derivative as yet has been converted to thebaine, and it is this conversion we now wish to report.

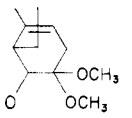
Ketalization of codeinone (I) with trimethyl orthoformate, methanol, and sulfuric acid was expected to give codeinone dimethyl ketal (II) or neopinone dimethyl ketal (III), but the only isolable product was 8-methoxy-Δ^6-dihydrothebaine (IV) [m.p. 190–191°; $[\alpha]^{25}D$ −133° (*c*, 0.9, ethanol); *anal.* Calcd. for $C_{20}H_{25}O_4N$: C, 70.0; H, 7.3; OCH_3, 27.1. Found: C, 70.2; H, 7.1; OCH_3, 27.5]. The structure of IV was established as follows. Degradation of the *methiodide* [m.p. 212–213°; $[\alpha]^{25}D$ −86° (*c*, 1, ethanol); *anal.* Calcd. for $C_{21}H_{28}O_4NI\cdot H_2O$: C, 50.1; H, 6.0; I, 25.2. Found: C, 50.3; H, 5.8; I, 24.8] gave a *methine* [m.p. 124–125°, $[\alpha]^{25}D$ −119° (*c*, 0.9, ethanol); *anal.* Calcd. for $C_{21}H_{27}O_4N$: C, 70.6; H, 7.6; OCH_3, 26.0. Found: C, 70.4; H, 7.5; OCH_3, 25.6] which was stable to all attempts at alkaline isomerization, indicating the alicyclic double bond was not Δ^7. With cyanogen bromide an *N-cyanonor* compound [m.p. 228–231°; $[\alpha]^{25}D$ −185° (*c*, 1, pyridine); *anal.* Calcd. for $C_{20}H_{22}O_4N_2$: C, 67.8; H, 6.3; N, 7.9. Found: C, 68.0; H, 6.4; N, 7.9] was obtained, indicating the double bond was not Δ^8. Acid hydrolysis gave a mixture of codeinone and 8-methoxydihydrocodeinone [m.p. 195–197°; *anal.* Calcd. for $C_{19}H_{23}O_4N$: C, 69.3; H, 7.0; OCH_3, 18.9. Found: C, 69.4; H, 6.8; OCH_3, 19.4], and hydroxylation with osmium tetroxide gave 7-hydroxy-8-methoxydihydrocodeinone, characterized as the *oxime* [m.p. 251–253°; $[\alpha]^{25}D$ −204 (*c*, 1, pyridine); *anal.* Calcd. for $C_{19}H_{24}O_5N_2$: C, 63.3; H, 6.7; N, 7.8; OCH_3, 17.2. Found: C, 62.9; H, 6.9; N, 7.9; OCH_3, 16.9].



I

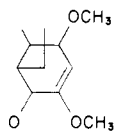


II

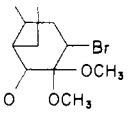


III

An alternative method for preparing codeinone dimethyl ketal was found in the dehydrobromination, using potassium *t*-amylate, of 7-bromodihydrocodeinone dimethyl ketal (V), itself prepared by methyl hypobromite addition to Δ^6-dihydrothebaine.[2] That the product was codeinone dimethyl ketal (II) [m.p. 138–139°; $[\alpha]^{21}D$ −233° (*c*, 0.5, ethanol); *anal.* Calcd. for $C_{20}H_{25}O_4N$: C, 70.0; H, 7.3; OCH_3, 27.1. Found: C, 69.8; H, 7.3; OCH_3, 27.2] derives from the following reactions. Acid hydrolysis gave codeinone, while hydrogenation led to dihydrocodeinone dimethyl ketal [m.p. 122–123°; $[\alpha]^{25}D$ −151° (*c*, 0.9, ethanol); *anal.* Calcd. for $C_{20}H_{27}O_4N$: C, 69.6; H, 7.9; OCH_3, 26.9. Found: C, 69.7; H, 8.0; OCH_3, 26.5], identical with the product formed from dihydrocodeinone and trimethyl orthoformate, methanol, and acid. The *methiodide* (m.p. 193–195°) was degraded to a $\Delta^{7,9}$-*methine* [m.p. 71–72°; $[\alpha]^{25}D$ −328° (*c*, 0.9, ethanol); $\lambda_{max}^{ethanol}$ 274 mμ, ε 8,500; *anal.* Calcd. for $C_{21}H_{27}O_4N$: C, 70.6; H, 7.6; OCH_3, 26.0. Found: C, 70.7; H, 7.5; OCH_3, 26.2] which was isomerized with alcoholic alkali to a $\Delta^{8(14),9}$-*methine* [oil; $[\alpha]^{25}D$ +301° (*c*, 1.2, ethanol); $\lambda_{max}^{ethanol}$ 318 mμ, ε 9,000; *anal.* Found: C, 70.6; H, 7.5; OCH_3, 25.8].



IV



V

When codeinone dimethyl ketal was treated with a dried solution of *p*-toluenesulfonic acid in chloroform, there was obtained a 40% yield of thebaine which after crystallization and sublimation was identical in m.p. and mixed m.p. (192–194°) and ultraviolet spectrum ($\lambda_{max}^{ethanol}$ 283 mμ, ε 7,500; λ_{min} 256 mμ, ε 3,700) with an authentic sample.

In a formal sense, this may be considered to constitute a synthesis of thebaine, since the Δ^6-dihydrothebaine used in the preparation above can be prepared from dihydrocodeinone,[3] and this in turn is easily made from codeine,[1,4] which has been synthesized.[5] Since thebaine recently has been converted to neopine,[6] the latter also may be considered as synthesized.

(1) L. F. Small and R. E. Lutz, "Chemistry of the Opium Alkaloids," Suppl. 103, Public Health Reports, U. S. Government Printing Office, Washington, D. C., 1932; K. W. Bentley, "The Chemistry of the Morphine Alkaloids," Clarendon Press, Oxford, England, 1954.

(2) We are greatly indebted to Dr. Lyndon F. Small for the details of this reaction. Our bromoketal melted at 116–117°, in agreement with Dr. Small's value.

(3) A. H. Homeyer, *J. Org. Chem.*, **21**, 370 (1956).

(4) H. Rapoport, R. Naumann, E. R. Bissell and R. M. Bonner, *ibid.*, **15**, 1103 (1950).

(5) M. Gates and G. Tschudi, THIS JOURNAL, **78**, 1380 (1956).

(6) H. Conroy, *ibid.*, **77**, 5960 (1955).

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RECEIVED AUGUST 27, 1956

A NEW ALKYLATION OF CARBONYL COMPOUNDS. II[1]

Sir:

We have submitted the new reaction we recently described for the alkylation and acylation of ketones

(1) Part I: G. Stork, R. Terrell and J. Szmuszkovicz, THIS JOURNAL, **76**, 2029 (1954).