ml. each being collected. Four peaks of activity were found. The second peak, fractions 35–40, 16,000 c.p.m., was fractionated on a Dowex-50 column by the procedure of Hirs, Moore and Stein.[9] Ammonium formate buffers at *p*H 2.21, 2.80 and 3.92 failed to elute radioactive material, but activity was eluted at *p*H 5.75 using ammonium acetate buffer. The buffer salts were removed, and the metabolite recrystallized from alcohol–ether as the hydrochloride. A total of 16 mg. was obtained, melting at 196–198°, and having approximately 300 c.p.m. per mg.

Anal. Calcd. for $C_6H_9N_2O_2Cl$: C, 40.81; H, 5.14; N, 15.86; Cl, 20.08. Found: C, 40.98; H, 5.31; N, 15.59; Cl, 20.19.

Preparation of N-Methylimidazoleacetic Acid Hydrochlorides (I and II).—1-Methyl-5-carbomethoxyimidazole[10] was converted to 1-methyl-5-cyanomethylimidazole picrate by the method of Jones and McLaughlin.[11] The picrate was converted to the free base, and hydrolyzed by the method of Pyman,[4] the 1-methylimidazole-5-acetic acid being isolated as the picrate, m.p. 180–181°. The same picrate, together with the picrate of 1-methylimidazole-4-acetic acid, m.p. 189–190°, was also prepared by the methylation of cyanomethylimidazole by Pyman's procedure,[4] followed by fractional crystallization of the methylcyanomethylimidazole picrates.

The picrates were converted to the hydrochlorides by passage of their aqueous solutions through a column of Dowex-1 in the chloride form, evaporation of the eluate to dryness, and recrystallization from ethanol–ether.

1-Methylimidazole-5-acetic acid hydrochloride melted at 204–206°.

Anal. Calcd. for $C_6H_9N_2O_2Cl$: Cl, 20.08. Found: Cl, 20.18.

1-Methylimidazole-4-acetic acid hydrochloride melted at 207–208°.

Anal. Calcd. for $C_6H_9N_2O_2Cl$: Cl, 20.08. Found: Cl, 20.04.

Paper Chromatography of the Metabolite.—All the paper chromatograms of the metabolite were run at room temperature by the ascending procedure on Schleicher & Schüll No. 604 paper. Spots of I and II, or mixtures of the two, as controls, were placed on the paper as 1% solutions. The paper was run overnight in the desired solvent, dried at room temperature in the hood for 0.5 hour, sprayed with a 0.05% solution of brom cresol green in alcohol, and redried at room temperature for 0.5 hour.

CHICAGO, ILLINOIS

(9) C. H. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **195**, 669 (1952).

(10) R. G. Jones, THIS JOURNAL, **71**, 644 (1949).

(11) R. G. Jones and K. C. McLaughlin, *ibid.*, **71**, 2444 (1949).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

10-Hydroxymorphine[1]

BY HENRY RAPOPORT AND SATORU MASAMUNE

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Cold chromic acid oxidation has been applied to several additional alkaloids of the morphine group. Δ^7-Desoxycodeine and neopine were converted to the corresponding 10-hydroxy compounds, while thebaine gave 14-hydroxycodeinone. Heroin under these conditions was uneffected, but O^3-allylmorphine was successfully oxidized, and after ether cleavage with sodium and liquid ammonia gave 10-hydroxymorphine. The *pK*'s and partition coefficients of morphine and 10-hydroxymorphine are compared.

The oxidation of codeine and a number of its derivatives[2,3] by cold chromic acid led to a series of compounds all of which were hydroxylated at the 10-position. Interest in these 10-hydroxy compounds stems from their possible occurrence as metabolic products of the morphine alkaloids and also from their possible biological activity, since introduction of the 10-hydroxyl group causes marked changes in the *pK* and solubility of these compounds. For these reasons, we have been interested in applying this hydroxylation reaction to further compounds in the morphine series, in particular to morphine.

The present report is concerned with the application of this oxidation to morphine, thebaine, neopine and Δ^7-desoxycodeine. The procedure was the same as that used previously and appears to be quite general. It consists in slowly adding a chromic acid–sulfuric acid solution to a cold solution of the alkaloid in dilute sulfuric acid. Quantities are adjusted so that there is very little further oxidation to the ketone, and the resulting mixtures of starting material and 10-hydroxy compound usually are separated through the increased water solubility of the latter.

Before this oxidation procedure could be applied to morphine, it was necessary to find a suitable protecting group for the phenolic hydroxyl in order to prevent oxidation of the aromatic ring. In addition to being stable to acid and oxidation, the conditions of the reaction, such a group subsequently must be removable under mild conditions since the hydroxylated molecule would now contain a secondary benzilic alcohol, a secondary allylic alcohol and an alicyclic double bond.

Cleavage of the 3-methyl ether with pyridine hydrochloride has been shown[4] to proceed in reasonable yield without affecting the allylic alcohol. To test whether the benzylic hydroxyl would survive this treatment, 10-hydroxy-Δ^7-desoxycodeine was heated with pyridine hydrochloride. However, in this case the conditions were apparently too drastic and led to a chlorine-containing, resinous product.

Protection was then sought through use of diacetylmorphine (heroin). Although heroin was stable to the conditions of acidity used in the oxidation, it was also stable to oxidation, and the only product isolated was heroin in 85% recovery. This indicated that esterification of the phenolic hydroxyl had deactivated the benzylic position and that an ether-type protecting group was required. Formation of benzyl ethers has frequently been used for this purpose since the benzyl group can be removed

(1) Supported by a grant from the National Institutes of Health, Bethesda, Maryland.

(2) H. Rapoport and G. W. Stevenson, THIS JOURNAL, **76**, 1796 (1954).

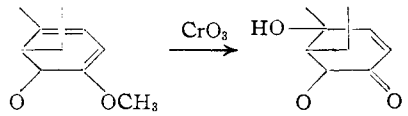
(3) H. Rapoport and Satoru Masamune, *ibid.*, **77**, 4330 (1955).

(4) H. Rapoport, C. H. Lovel and B. M. Tolbert, *ibid.*, **73**, 5900 (1951).

by such reagents as lithium aluminum hydride,[5] sodium in liquid ammonia,[5] or the Grignard reagent.[6] However, when the 3-benzyl ether of morphine was subjected to the general oxidation procedure, a strong odor of benzaldehyde was apparent and no 10-hydroxylated material could be isolated.

A successful oxidation was finally achieved with the 3-allyl ether of morphine, but the conversion to 10-hydroxy compound and the over-all recovery of alkaloidal material was much poorer than with the codeine derivatives, apparently due to competing oxidation of the allyl ether group. Sodium in liquid ammonia then smoothly cleaved the allyl ether and 10-hydroxymorphine was obtained after purification as the triacetyl derivative.

Further application of this hydroxylation reaction then was made to neopine, Δ^7-desoxycodeine and thebaine. With the first two compounds, the expected reaction occurred and the corresponding 10-hydroxy compounds were obtained in moderate yield. With thebaine, which was found to be stable to the acid conditions employed in the oxidation, the reaction took an entirely different course. The chromic acid was consumed much more rapidly than in any of the previous examples, and the product, rather than 10-hydroxylated material, was 14-hydroxycodeinone which has been prepared previously by hydrogen peroxide oxidation of thebaine.[7]



Since 10-hydroxymorphine was of particular interest from the biological standpoint, some of its physical properties were determined for comparison with morphine.[8] The pK_a's of 10-hydroxymorphine were found to be 7.13 (pK_1, ammonium ion) and 9.06[9] (pK_2, phenol). The corresponding values for morphine are pK_1 8.05 and pK_2 9.29.[9,10] At its isoelectric point (pH 8.09), 10-hydroxymorphine has an apparent partition coefficient between chloroform and water of 1.00 whereas for morphine at its isoelectric point (pH 8.67) the value is 0.46. This was surprising since with every previous compound introduction of the 10-hydroxyl had significantly increased the water solubility and decreased the solubility in organic solvents.

In order to determine whether this apparent inconsistency was due to the relative amounts of charged forms present in the two cases, the true partition coefficient for each compound was calculated. Since at the isoelectric point, there are equal amounts of ammonium ion and phenolate ion forms present, the concentration of singly charged species can be calculated from the experimental K_1 and K_2. The neutral form consists of both undissociated molecules and zwitterions, and the constant for this equilibrium, K_z, was assumed to equal the ratio of K_2 to K_1 as a reasonable approximation. These calculations indicate that morphine at its isoelectric point is 68% neutral species of which 5% is zwitterion. For 10-hydroxymorphine at its isoelectric point there is 82% neutral species of which 1% is zwitterion. From these values and the assumption that only undissociated molecules dissolve in the chloroform phase, the true partition coefficient for 10-hydroxymorphine is found to be 1.23 and that for morphine is 0.72. Although consideration of the charged forms has brought the partition coefficients closer together, still introduction of the 10-hydroxyl group into morphine has increased rather than decreased its solubility in chloroform relative to that in water.

(5) W. H. Hartung and R. Simonoff in "Organic Reactions," Vol. VII, John Wiley and Sons, New York, N. Y., 1953, p. 263.

(6) M. S. Kharasch and R. L. Huang, *J. Org. Chem.*, **17**, 669 (1952).

(7) R. E. Lutz and L. Small, *ibid.*, **4**, 220 (1939).

(8) Most of these data were obtained by Dr. G. W. Stevenson of this Laboratory.

(9) Determined by potentiometric titration.

(10) Previously reported as 9.85 by I. M. Kolthoff, *Biochem. Z.*, **162**, 289 (1925).

Experimental[11]

General Oxidation Procedure.—All oxidations followed the general procedure previously used[2,3] and consisted in adding a solution of chromic acid in 10 N sulfuric acid to a well-stirred solution of the alkaloid in 1 N sulfuric acid maintained at 3–5°. For 10 mmoles of alkaloid, 500 ml. of 1 N sulfuric acid, 0.66 g. of chromic acid and 33 ml. of 10 N sulfuric acid were used, and addition was made over a 6 to 8-hr. period from a capillary-tipped dropping funnel extending below the surface of the alkaloid solution. One hour after completion of the addition, sodium sulfite was added to destroy any excess oxidant, the solution was adjusted to pH 4–5 with sodium carbonate, and then it was made strongly alkaline with sodium hydroxide prior to extraction with a suitable solvent.

O^3-Benzylmorphine.—O^3-Benzylmorphine was prepared as previously described[12] and was purified by chromatographing on alumina using chloroform for elution. Crystallization from ethyl acetate gave an 85% yield of material of m.p. 127–128.5°.

When subjected to the standard oxidation procedure above, a strong odor of benzaldehyde was detected and the only isolable product was a 58% recovery of crude O^3-benzylmorphine.

O^3-Allylmorphine.—The procedure of Földi[13] was used and the reaction mixture, after being made alkaline, was extracted with benzene. Chloroform–benzene (1:1) eluted the O^3-allylmorphine from an alumina column in 90% yield, and the residue after evaporating the solvent was suitable for oxidation without further purification; $[\alpha]^{21}D$ −117° (c 1.4, ethanol).

O^3-Allyl-10-hydroxymorphine.—Oxidation of 12.7 g. of O^3-allylmorphine by the general procedure and extraction with chloroform left 8.2 g. of residue on evaporation of the chloroform. This material was applied to an alumina column (6.5 × 8.0 cm.) using benzene–chloroform (7:3) and by gradually changing the solvent to 1:1, 3.6 g. (28%) of starting material was recovered. Continued elution with chloroform gave 1.9 g. of the 10-hydroxy compound which was crystallized from ethyl acetate and sublimed at 120° (10 μ); yield 1.3 g. (10%), m.p. 137.5–139°, $[\alpha]^{22}D$ −100° (c 0.85, ethanol).

Anal. Calcd. for $C_{20}H_{23}O_4N$: C, 70.4; H, 6.8. Found: C, 70.2; H, 6.7.

10-Hydroxymorphine.—A solution of 700 mg. (2.05 mmoles) of O^3-allyl-10-hydroxymorphine in 30 ml. of liquid ammonia was treated with 230 mg. (10 mmoles) of sodium in portions with stirring. The vigorous gas evolution soon subsided and a permanent blue color developed within about 10 minutes, after which 0.7 ml. of methanol was added and the now colorless solution was evaporated under a nitrogen stream and then *in vacuo*. Solution of the residue in water (1.8 ml.), adjustment of the pH to 8.3 with hydrochloric

(11) All melting points are corrected and those above 200° were taken in evacuated capillaries; microanalyses were performed by the Microchemical Laboratory, University of California. The alumina used for chromatography was Merck, reagent grade.

(12) J. F. von Mering, U. S. Patent 584,388 (1897).

(13) Z. Földi, *Ber.*, **53**, 1839 (1920).

acid, and cooling gave 550 mg. of a yellow, crystalline precipitate which was extracted with acetone in a Soxhlet extractor for two days. The solid in the boiling flask plus the additional material obtained on concentrating the acetone was dissolved in 300 ml. of 50% aqueous ethanol and this solution was treated with 200 mg. of decolorizing carbon, filtered and concentrated to dryness.

The resulting 490 mg. (79% yield) of 10-hydroxymorphine was purified further through the triacetyl derivative. Heating a solution of 1 g. of 10-hydroxymorphine in 35 ml. of acetic anhydride and 5 ml. of pyridine for seven hours at 100° and evaporating the solution to dryness *in vacuo* left a residue which was chromatographed on alumina (acid washed, 3.5 × 6 cm.). The chromatogram was developed with benzene and the elution was completed with benzene–chloroform (8:2 and 1:1). Crystallization from ethyl acetate gave 1.16 g. (82% yield) of **triacetyl-10-hydroxymorphine**; m.p. 186–187°, $[\alpha]^{22}D$ −86.8° (*c* 0.93, ethanol).

Anal. Calcd. for $C_{23}H_{25}O_7N$: C, 64.6; H, 5.9. Found: C, 64.5; H, 6.1.

To obtain 10-hydroxymorphine, a solution of the triacetyl derivative in 0.5 *N* sodium hydroxide in 50% aqueous ethanol was heated under reflux overnight in a nitrogen atmosphere. The solution was concentrated *in vacuo* to one-sixth its volume, the *p*H was adjusted to 8.3, and the precipitated white, crystalline **10-hydroxymorphine** was filtered from the cooled solution in 93% yield. It was washed with water, ethanol and ethyl acetate and dried at 100° *in vacuo;* m.p. 325° with dec. after sintering at 230–240°; $[\alpha]^{21}D$ −94.5° (*c* 0.69, 2 *N* acetic acid).

Anal. Calcd. for $C_{17}H_{19}O_4N$: C, 67.8; H, 6.4. Found: C, 68.0; H, 6.7.

10-Hydroxyneopine.—Application of the general oxidation procedure to 20 g. of neopine resulted in a recovery of 16 g. of alkaloidal material from the basified reaction mixture by chloroform extraction. This material was then chromatographed on an alumina column (6 × 9 cm.) and 12.5 g. (62%) of neopine was recovered from the benzene–chloroform (7:3) eluate. 10-Hydroxyneopine (2.5 g.) was then eluted with chloroform, crystallized from acetone, and sublimed at 170° (10 μ) to give 2.1 g. (10%) of material; m.p. 204–205°, $[\alpha]^{22}D$ −8.4° (*c* 0.9, ethanol).

Anal. Calcd. for $C_{18}H_{21}O_4N$: C, 68.6; H, 6.7. Found: C, 68.2; H, 6.7.

10-Hydroxy-Δ^7-desoxycodeine.—A total of 10.1 g. of alkaloidal material was recovered by a three-day continuous benzene extraction of the basified reaction mixture from the chromic acid oxidation of 14.2 g. of Δ^7-desoxycodeine. This residue was dissolved in benzene and applied to an alumina column (6 × 7 cm.) from which benzene eluted 5.1 g. (36%) of recovered Δ^7-desoxycodeine and chloroform eluted 5.2 g. of crude hydroxy compound. Crystallization from ethyl acetate and sublimation at 125° (20 μ) resulted in 3.8 g. (25%) of 10-hydroxy-Δ^7-desoxycodeine; m.p. 144.5–145.5°, $[\alpha]^{21}D$ −67.1° (*c* 0.63, ethanol).

Anal. Calcd. for $C_{18}H_{21}O_3N$: C, 72.2; H, 7.1. Found: C, 72.4; H, 6.9.

Oxidation of Thebaine.—When the general oxidation procedure was applied to 10.4 g. of thebaine, the oxidant was consumed much more rapidly than in any of the previous oxidations. The reaction mixture, after being made alkaline, was extracted thoroughly with chloroform, and the chloroform was dried over magnesium sulfate and evaporated. Treatment of the residue (5.5 g.) in benzene with another portion of magnesium sulfate, which was a particularly effective decolorizing agent in this case, and evaporation of the benzene left colorless material which was crystallized from chloroform–ethanol. The crystalline material (0.9 g.) was 14-hydroxycodeinone, m.p. 273–274°, $[\alpha]^{25}D$ −110° (*c* 0.81, 10% acetic acid) [reported[7] m.p. 275–276°, $[\alpha]^{25}D$ −111° (*c* 0.90, 10% acetic acid)]. Application of the mother liquors residue to an alumina column (3 × 13 cm.) in benzene and elution with benzene–chloroform (9:1) gave an additional 1.6 g. of 14-hydroxycodeinone, m.p. 269–271°.

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[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Pinus Alkaloids. The Alkaloids of *P. sabiniana* Dougl. and Related Species

BY W. H. TALLENT, V. L. STROMBERG AND E. C. HORNING

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The presence of alkaloids in leaves of nine species of pines was indicated by qualitative tests. The possible relationship of these observations to Mirov's biochemical classification of pines is discussed. From leaves of *P. sabiniana* Dougl. two alkaloids were isolated: (+)-α-pipecoline and a new organic base, $C_9H_{17}N$, to which the name *pinidine* has been given.

The economic importance of members of the genus *Pinus* in many areas of the world has resulted in widespread study of the constituents of these trees. The volume of chemical literature dealing with terpenes and resin acids is indicative of the extensive effort which has been expended on *Pinus* compounds, and, although no specific studies on the presence or absence of organic bases in pines has been published, it has been generally assumed that the genus as a whole is alkaloid-free. This investigation was initiated as a consequence of an observation[1] that a pine leaf specimen gave positive alkaloid precipitation tests; this observation was confirmed and extended through an examination of twenty-seven species of pines. Small samples of fresh (undried) leaves and twigs were subjected to qualitative examination for the presence of alkaloids. Mayer's solution and silicotungstic acid solution were used as precipitating agents. The results are summarized in Table I. Four species gave positive tests with both reagents and five additional species gave tests with one reagent (silicotungstic acid). An alkaloid of unknown structure, *pinidine*, was found in *P. sabiniana*, *P. jeffreyi* and *P. torreyana*. These species gave moderately strong positive tests for alkaloids, while the other species gave weakly positive tests. These results are particularly interesting when they are compared with Mirov's[2] data for the classification of pines on the basis of turpentine constituents. Mirov placed *P. sabiniana*, *P. jeffreyi*, *P. torreyana* and *P. coulteri* in the group Macrocarpae because of the occurrence of saturated hydrocarbons in these pines; the inclusion of *P. jeffreyi* in this group by Mirov differs from Shaw's treatment[3] and is on the basis of this fact. The data in Table I extend the biochemical similarity of these plants and suggest

(1) M. E. Wall, C. S. Fenske, J. J. Willaman, D. S. Correll, B. G. Schubert and H. S. Gentry, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 438 (1955).

(2) N. T. Mirov, *Z. Forstgenetik und Forstpflanzenzuchtung*, **2**, 93 (1953).

(3) G. R. Shaw, Arnold Arboretum Pub. No. 5 (1914).