duced pressure. After two recrystallizations from methanol, the residue gave cholestanyl acetate, m.p. 108–109°, which gave no depression of the melting point when mixed with an authentic sample. Its infrared spectrum was undistinguishable from that of the reference material.

3β-Acetoxy-5,22-choladiene (IIIb). A solution of 5.0 g. of triphenylphosphine in 50 ml. of anhydrous toluene was heated in a pressure flask with 10.2 g. of freshly distilled ethylbromide at 105° for 24 hr. The precipitate was filtered and dried; 6.35 g., m.p. 202–205°, reported 202–205°[17]; λ_{max} 6.90, 6.99, and 10.03μ.

A solution of 1.68 g. of 3β-acetoxybisnor-5-cholen-22-al (I)[15] in 25 ml. of anhydrous ether was added to the ylide generated from 3.35 g. of the triphenylethylphosphonium bromide and 7.0 ml. of 1.3*N* butyllithium in 25 ml. of absolute ether in a pressure flask. The mixture was then treated as described above, and the crude acetate was chromatographed on silicic acid: Celite and recrystallized from methanol; 0.74 g. m.p. 117–122°. The infrared spectrum showed a strong band at 10.27μ indicative of a *trans*-oriented double bond,[8-10] and a weak band at 10.02μ indicative of a *cis* double bond.[11]

The mixed acetates were refluxed with 100 ml. of benzene and 0.35 g. of iodine for 6 hr. The solution was cooled, washed with a solution of sodium thiosulfate, dried, and evaporated to dryness. The residue was dissolved in hexane, and the solution chromatographed over neutral alumina (Brockmann II). Hexane eluted a small fraction containing halogen. The main fraction was eluted with hexane-benzene (9:1). It was recrystallized three times from methanol; m.p. 127–129°, $(\alpha)_D^{26}$ −72.2° (C = 0.64 in $CHCl_3$); λ_{max} 5.76, 8.02, and 10.27 μ in KBr.

Anal. Calcd. for $C_{26}H_{40}O_2$: C, 81.20; H, 10.46. Found: C, 80.85; H, 10.56.

$\Delta^{5,22}$-Choladien-3β-ol (IIIa). Hydrolysis of the acetate with potassium hydroxide in ethanol gave the sterol which was recrystallized from methanol; m.p. 117–117.5°; $(\alpha)_D^{25}$ −65.8°; (C = 0.59 in $CHCl_3$); λ_{max} 2.96, 10.28, and 12.50μ in KBr.

Anal. Calcd. for $C_{24}H_{38}O$: C, 84.15: H, 11.18. Found: C, 83.94; H, 11.25.

(17) G. Wittig and D. Wiggenberg, *Ann.*, 606, 1 (1957).

NEW HAVEN, CONN.

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Hydroxylated Codeine Derivatives

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The application of osmium tetroxide to the introduction of hydroxyl groups in the codeine and neopine series is described. Lithium aluminum hydride or sodium borohydride reduction of 8,14-dihydroxydihydrocodeinone leads to epimeric dihydroxydihydrocodeines. Analogous treatment of 14-hydroxycodeinone yields *true* 14-hydroxycodeine.

The observation that 3,4 dimethoxy-$\Delta^{6,7}$-13-ethylhexahydrophenanthrene (a degradation product of dihydrothebaine)[3] reacted smoothly with osmium tetroxide to yield, after hydrolysis of the osmate ester, the corresponding 6,7-glycol, led to the present study of the action of this reagent on the following morphine derivatives containing an alicyclic unsaturated center in ring C: desoxycodeine-C, codeine methyl ether, acetyl codeine, acetyl isocodeine, and acetyl neopine. It was of interest to pursue this investigation for several reasons: (1) the possibility of arriving at pharmacologically interesting substances was apparent; (2) conceivably this approach could improve upon earlier hydroxylation attempts (of codeine) where low yields were reported[4]; and (3) a route to the unknown 8,14-dihydroxylated neopine[5] (VI) might be afforded.

The considerable number of codeine and codeinone derivatives (containing one or more new hydroxyl groups in ring C) together with their ED_{50} values, relative to codeine, are shown in Table I. It will be noted that enhanced activity is elicited principally by those substances derived from 14-hydroxycodeinone VII. None of the presently reported derivatives showed significant analgesic activity; with the exception of 7-hydroxydihydroco-

TABLE I

Compound	ED_{50} (Mice)[a]
10-Hydroxycodeine	50.4
14-Hydroxycodeine	17.2
10-Hydroxydihydrocodeine	22.5
14-Hydroxymorphinone	42.2
14-Hydroxydihydromorphinone	0.17
14-Hydroxydihydromorphine	1.05
14-Hydroxydihydrocodeinone	1.4
8-Hydroxydihydrocodeinone	>150
14-Hydroxycodeinone	6.1
8,14-Dihydroxydihydromorphinone	6.3
8,14-Dihydroxydihydrocodeinone	16.7
7-Hydroxydihydrocodeine	None[b]
7,8-Dihydroxydihydrocodeine	>400
7,8-Dihydroxydihydrocodeine methyl ether	>100
8,14-Dihydroxydihydrocodeine	>200

(1) Koppers Co., Verona, Pa.

(2) Deceased, June 1957.

(3) L. J. Sargent and L. F. Small, *J. Org. Chem.*, 16, 1031 (1951).

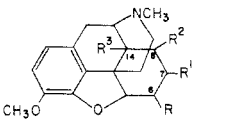
(4) R. S. Cahn and R. Robinson, *J. Chem. Soc.*, 908 (1926).

(5) K. W. Bentley, *The Chemistry of the Morphine Alkaloids*, Oxford University Press, London, ref. 5, p. 124 (1954).

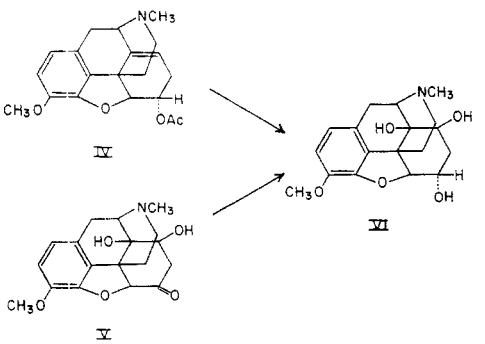
[a] We are indebted to Dr. Nathan B. Eddy, of this Laboratory, for permission to use these unpublished data. Codeine ED_{50} = 14.2. (ED_{50} is the dose which is effective for 50% of the test subjects.) [b] Fatal dose (LD_{50} = 50).

deine which was relatively toxic (LD_{50} = 50 mg./kg.), their ED_{50} values were above 100 mg./kg.

The first hydroxycodeine to be described, now known[6] to be 10-hydroxycodeine, was prepared by Knorr[7] through the action of cold chromic acid on codeine. Subsequently Freund[8] and Wieland[9] synthesized 1- or 2-hydroxycodeine from nitrocodeine, and Cahn[4] obtained 7,8-dihydroxydihydrocodeine (III) in low yield from the permanganate oxidation of codeine. 14-Hydroxycodeinone (VII), from the reaction of thebaine with hydrogen peroxide,[10] should also be mentioned, whose reduction product, 14-hydroxydihydrocodeinone (Oxycodone[11a]) and its demethylated derivative, 14-hydroxydihydromorphinone (Oxymorphone[11a, b]) show extraordinary analgesic powers. The oxidation of thebaine by manganic acetate, first studied by Vieböck,[12] yields 8-(or 14-)acetyl-8,14-dihydroxydihydrothebaine which, upon brief treament with hot dilute HCl, gives 8,14-dihydroxyhydrocodeinone (V). In a careful study of the catalytic hydrogenation of 14-hydroxycodeinone (VII), Lutz[13] found that two isomers were formed, *viz:* 14-hydroxydihydrocodeine-B (IX) along with a lesser amount of 14-hydroxydihydrocodeine-C (the two being epimeric about C[6]). Both differed, however, from 14-hydroxydihydrocodeine-A which resulted from catalytic hydrogenation of the zinc-acetic acid[14] reduction product of 14-hydroxycodeinone (VII). It was suggested[13] that the singularity of isomer-A was possibly due to a structural alteration that occurred during the metal-acid treatment, a view which is supported by pharmacological studies. Thus, isomers B and C differ in their analgesic activity to about the extent anticipated for an epimeric pair, whereas isomer-A deviates widely, in its effect, from the other two.



I - R = R′ = OH; $R^2 = R^3 = H$
II - R = OCH_3; R′ = R^2 = OH; R^3 = H
III - R = R′ = R^2 = OH; R^3 = H



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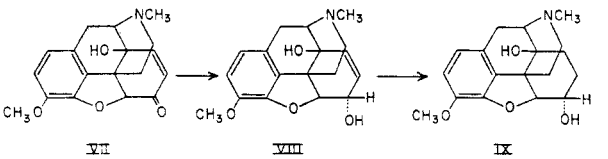
VI

V

(6) H. Rapoport and G. W. Stevenson, *J. Am. Chem. Soc.*, **76**, 1796 (1954).
(7) F. Ach and L. Knorr, *Ber.*, **36**, 3067 (1903).
(8) M. Freund and E. Speyer, *Ber.*, **44**, 2339 (1911).



VII VIII IX

More recently Findlay[15] discovered that codeinone (in *acid* solution) is capable of adding the elements of water across its 7,8-unsaturated center to form 8-hydroxydihydrocodeinone which upon reduction gave rise to 8-hydroxydihydrocodeine. A remarkable and related observation is that due to Weiss[16] who found that 14-hydroxymorphinone (in *alkaline* medium) readily added the elements of water to yield 8,14-dihydroxydihydromorphinone. Rapoport[17] has latterly reported the use of osmium tetroxide in a series of hydroxylation experiments with codeine derivatives having $\Delta^{7,8}$ unsaturation and in the one instance, 7,8-dihydroxydihydrocodeine (III), where his and our experiments overlap, good agreement in physical properties is evident.

The hydroxylation of the various morphine derivatives outlined below was carried out as described by us previously.[3] However, in cleaving the osmate esters, we found it advantageous, from the standpoint of product work-up, to substitute sodium sulfite[18] for the alkaline mannitol procedure utilized earlier.[3]

Apart from the straightforward osmic acid oxidations referred to, this investigation also afforded the opportunity of verifying the purported *cis* hydroxyl arrangement in Vieböck's[12] 8,14-dihydroxydihydrocodeinone (V). Reduction of the carbonyl function in the latter either with lithium aluminum hydride or sodium borohydride resulted in a mixture of crystalline, epimeric 8,14-dihydroxydihydrocodeines (VI) one of which was identical with the osmic acid oxidation product of acetylneopine (IV) in which only a *cis* (8,14-)glycol arrangement is tenable. The hydroxyl group in the other epimer presumably has the *iso* configuration at C.[6] Of the two reduction techniques tested, lithium aluminum hydride proved superior on two counts: (1) workup

(9) H. Wieland and P. Kappelmeier, *Ann.*, **382**, 306 (1911).
(10) M. Freund and E. Speyer, *J. prakt. Chem.*, **94**, 135 (1916).
(11) (*a*) International non-proprietary name; (*b*) U. Weiss, *J. Am. Chem. Soc.*, **77**, 5891 (1955).
(12) F. Vieböck, *Ber.*, **67**, 197 (1934).
(13) R. E. Lutz and L. F. Small, *J. Org. Chem.*, **4**, 220 (1939).
(14) E. Speyer and K. Sarre, *Ber.*, **57**, 1404 (1924).
(15) S. P. Findlay and L. F. Small, *J. Am. Chem. Soc.*, **72**, 3247 (1950); **73**, 4001 (1951).
(16) U. Weiss, *J. Org. Chem.*, **22**, 1505 (1957).
(17) H. Rapoport, M. S. Chada, and C. H. Lovell, *J. Am. Chem. Soc.*, **79**, 4694 (1957); *cf.* ref. 11a in latter. (*Note:* With the exception of the neopine glycols, all of the substances presently reported were prepared prior to 1951).
(18) (*a*) A. Butenandt, J. Schmidt-Thomé, and H. Paul, *Ber.*, **72**, 1112 (1939); (*b*) L. H. Sarett, *J. Am. Chem. Soc.*, **71**, 1171 (1949).

was less cumbersome and (2) better over-all yields were obtained.

Evidence in support of the *normal* configuration of the C^{14}-hydroxyl in 8,14-dihydroxydihydrocodeinone (V) was forthcoming from an examination of the optical rotation of the phenylhydrazone of this substance. The observed value, $[\alpha]_D^{20}$ −604.8° is in accord with Gates's[19] consistent findings of abnormally high rotations by dinitrophenylhydrazones in the *normal* (*cis*) series of the morphine alkaloids. The phenylhydrazones of 14-hydroxycodeinone and of 14-hydroxydihydrocodeinone were prepared for comparison and these showed $[\alpha]_D^{20}$ values of −1416° and −649°, respectively.

That 14-hydroxydihydrocodeine-A differs from the epimers 14-hydroxydihydrocodeine-B and -C has already been alluded to. Because of the dubiety of the A-isomer and its precursor "14-hydroxycodeine,"[20] *true* 14-hydroxycodeine (VIII) was prepared, for the first time, by sodium borohydride reduction of 14-hydroxycodeinone (VII). Since Gates[21] has recently shown that this reagent reduces codeinone practically quantitatively and stereospecifically to codeine, it is reasonable to infer that *true* 14-hydroxycodeine has the C^6 codeine configuration. Catalytic reduction of *true* 14-hydroxycodeine yielded a substance which was identical with 14-hydroxycodeine-B; this must therefore have the C^6 codeine configuration too.[22] Since 14-hydroxydihydrocodeine-B and -C were shown[13] to be epimeric about C^6, it follows that the C-isomer must have the isocodeine configuration at this center.

EXPERIMENTAL[23]

7-Hydroxydihydrocodeine (I).[24] To a cold (0°) solution of 0.292 g. of osmium tetroxide (1 equiv.) and 0.195 ml. (2 equiv.) of dry pyridine in 15 ml. of dry ether, a cold solution of 0.325 g. of desoxycodeine-C in 15 ml. of dry ether was added at one time. After keeping at 0° for 5 hr., the brown adduct was collected, washed with ether, and dried; yield 0.68 g. (85%).

A mixture of the adduct with 2.4 g. of sodium sulfite, 16 ml. of 95% ethanol, and 10 ml. of water was heated (reflux) for 3.5 hr. During this interval a black precipitate gradually separated. The latter was centrifuged and washed with two 10-ml. portions of boiling methanol. Concentration (vacuum) of the combined supernate and washings afforded a crystalline residue which was washed with a little ice-cold water and dried; yield 0.19 g. (55%), m.p. 227–229°. After three crystallizations from methanol, there were obtained rectangular plates, m.p. 230–232°. The analytical sample was dried for 2.5 hr. at 78°/0.4 mm.

Anal. Calcd. for $C_{18}H_{23}NO_4$: C, 68.1; H, 7.30; N, 4.41. Found: C, 68.2; H, 7.45; N, 4.56. $[\alpha]_D^{20}$ −138° (c = 0.333, methanol).

7,8-Dihydroxydihydrocodeine methyl ether (II). Codeine methyl ether (6.14 g.) in 100 ml. of cold anhydrous ether was added to a cold solution of 5 g. of osmium tetroxide in 200 ml. of anhydrous ether. The system was refrigerated for 6 hr. and then kept at room temperature for 72 hr. After removal of the solvent (vacuum), the brown residue was heated (reflux) for 3.5 hr. with 50 g. of sodium sulfite in 200 ml. of water and 125 ml. of ethanol. Centrifugation was carried out as above and the combined supernate and ethanol washings were removed (vacuum) to yield 5 g. of a colorless, crystalline product. The latter was recrystallized three times from dilute ethanol (Norit) to give 4 g. (60%) of glycol, m.p. 216–218°.

Anal. Calcd. for $C_{19}H_{25}NO_5$: C, 65.69; H, 7.25. Found: C, 65.67; H, 6.97. $[\alpha]_D^{20}$ −74.8° (c = 1.01, 95% ethanol).

7,8-Diacetoxydihydrocodeine methyl ether. A solution of 0.3 g. of II in a mixture of 6 ml. of acetic anhydride and 10 ml. of anhydrous pyridine was kept at 5° for 96 hr. Removal of the solvent (vacuum) afforded a quantitative yield of the diacetoxy derivative which proved to be quite soluble in ether, ethanol, or acetone. After three sublimations at 125°/0.2 mm., 0.25 g. of pure derivative, m.p. 133–135°, was obtained.

Anal. Calcd. for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.78. Found: C, 64.09; H, 6.75.

7,8-Dihydroxydihydrocodeine (III).[25] A solution of 6.68 g. of acetylcodeine in 100 ml. of cold, anhydrous ether was added to one of 5 g. of osmium tetroxide in 150 ml. of the same solvent, and the mixture was kept at room temperature for 72 hr. After the usual work-up, the aqueous solution (following removal of ethanol) was extracted with 4 × 25 ml. portions of chloroform from which 5.6 g. of crude III was obtained. Two crystallizations from ethanol (Norit) yielded 3.9 g. (60%) of III, m.p. 207–208° (lit.[4,17] m.p. 208–209°; 210–211°).

Anal. Calcd. for $C_{18}H_{23}NO_5$: C, 64.9; H, 6.9. Found: C, 65.0; H, 7.0.

The *triacetoxy* derivative prepared as above (acetic anhydride–pyridine) was crystallized twice from ether and sublimed at 186°/0.1 mm.; m.p. 198–199° (lit.[4,17] m.p. 200°; 200–202°).

Anal. Calcd. for $C_{24}H_{29}NO_8$: C, 62.7; H, 6.36. Found: C, 62.7; H, 6.35.

Acetyl neopine (IV).[26] Five g. of neopine was added to a cooled mixture of 20 ml. of dry pyridine and 10 ml. of acetic anhydride. After keeping at 25° for 48 hr. and the usual work-up, the product was evaporatively distilled (cold finger) at 125–130°/0.3 mm.; yield 4.9 g. colorless crystals. A sample was resublimed; m.p. 117°.

Anal. Calcd. for $C_{20}H_{23}NO_4$: CH_3CO, 12.6. Found: CH_3CO, 12.5. $[\alpha]_D^{20}$ + 15.2° (c = 0.972, 95% ethanol).

8,14-Dihydroxydihydrocodeine (VI). The interaction of IV (2.7 g.) in 50 ml. of dry ether with 2 g. of osmium tetroxide in 50 ml. of the same solvent gave, after 48 hr., 1.6 g. of slightly tacky crystals. Two crystallizations from ethyl acetate yielded 1.3 g. (50%) of colorless prisms which sublimed unchanged at 150°/0.2 mm., m.p. 186–186.5° (evac. tube).

Anal. Calcd. for $C_{18}H_{23}NO_5$: C, 64.8; H, 7.0. Found: C, 65.1; H, 6.84. $[\alpha]_D^{20}$ −147° (c = 1.07, 95% ethanol).

Acetylisocodeine. The acetylation of isocodeine (10 g.) was carried out, as above, with a mixture of 10 ml. of dry

(19) M. Gates and G. Tschudi, *J. Am. Chem. Soc.*, **78**, 1380 (1956); especially ref. 23 therein.

(20) *Cf.* ref. (14). An attempt to elucidate this structure is in progress.

(21) M. Gates, *J. Am. Chem. Soc.*, **75**, 4340 (1953).

(22) *Cf.* ref. (13). These authors intimated that their pharmacological data suggested C^6-dihydrocodeine and dihydroisocodeine configurations, respectively, for the B and C isomers.

(23) Analyses are by the Analytical Service Laboratory of this Institute, under the supervision of Dr. W. C. Alford. Melting points are uncorrected.

(24) K. Goto and T. Arai, *Bull. Chem. Soc. Japan*, **17**, 113 (1942), obtained this substance (30% yield) through permanganate oxidation of desoxycodeine-C and reported the empirical formula $C_{18}H_{23}NO_4$–0.5 H_2O, which possibly accounts for their lower $[\alpha]_D$ −128°.

(25) *Cf.* refs. (4) and (17).

(26) C. F. van Duin, R. Robinson, and J. C. Smith, *J. Chem. Soc.*, 903 (1926) first reported this substance as an oil.

pyridine and 10 ml. of acetic anhydride. The colorless crystals were purified from absolute ethanol (Norit) at −5° (to minimize losses); yield 8 g. of small prisms, m.p. 88–89°.

Anal. Calcd. for $C_{20}H_{23}NO_4$: CH_3CO, 12.6; Found: CH_3CO, 12.4. $[\alpha]_D^{20}$ −270° (c = 1.02, 95% ethanol).

7,8-Dihydroxydihydroisocodeine. In the manner outlined above, acetylisocodeine (2.5 g.) in 50 ml. of dry ether was oxidized (during 24 hr.) with 2 g. of osmium tetroxide in 75 ml. of the same solvent. After concentration, hydrolysis and the customary manipulation, 1.9 g. of an amber oil (that crystallized overnight) was obtained. This was recrystallized from absolute ethanol (Norit); yield 1.1 g. (42%) of nearly colorless crystals. A sample was recrystallized again and dried 1 hr. at 70°/0.1 mm., m.p. 241–242° (evac. tube); it was not completely anhydrous.

Anal. Calcd. for $C_{18}H_{23}NO_5 \cdot 0.5\ H_2O$: C, 63.2; H, 7.07; H_2O, 2.63. Found: C, 63.8; H, 6.86; H_2O, 2.30. $[\alpha]_D^{20}$ −78.7° (c = 0.502, 95% ethanol).

8,14-Dihydroxydihydrocodeinone (V). The hydroxylation of thebaine (40 g.) with freshly prepared manganic acetate according to the procedure outlined by Vieböck[12] yielded 7 g. of dihydroxydihydrocodeinone. After recrystallization from ether 5.7 g. of colorless prisms were obtained; m.p. 169–170° (lit. m.p. 170°). The substance sublimes unchanged at 165–170°/0.05 mm.

Anal. Calcd. for $C_{18}H_{21}NO_5$: C, 65.2; H, 6.39; active H, 2.0. Found: C, 65.3; H, 6.32; active H, 2.2. $[\alpha]_D^{20}$ −191° (c = 1.0, 95% alcohol).

The *phenylhydrazone.* A mixture of 0.25 g. of V in 4.5 ml. of absolute ethanol with 0.25 g. of redistilled phenylhydrazine and 4 drops of glacial acetic acid was heated on the steam bath for 10 mins. Basification of the cooled solution (NH_4OH) precipitated a yellow gum which solidified after 48 hr. The powdered product was collected, washed with water, and dried; yield 0.25 g. This was dissolved in 1 ml. of hot methanol and seeded; the practically colorless plates were rinsed with a few drops of cold methanol. After a second recrystallization, the derivative showed the m.p. 162–164° dec. (evac. tube), with previous softening at 112°.

Anal. Calcd. for $C_{24}H_{27}N_3O_4$: N, 9.97. Found: N, 10.2. $[\alpha]_D^{20}$ −605° (c = 1.03, $CHCl_3$).

14-Hydroxycodeinone phenylhydrazone. From 0.5 g. of VII (as above) a gum was obtained which crystallized in glassy prisms (0.35 g.) from absolute ethanol, m.p. 193–194° (evac. tube).

Anal. Calcd. for $C_{24}H_{25}N_3O_3$: N, 10.4. Found: N, 10.2. $[\alpha]_D^{20}$ −1416° (c = 0.998, $CHCl_3$).

14-Hydroxydihydrocodeinone phenylhydrazone. This derivative, prepared as above, was recrystallized twice from absolute ethanol; faintly tan needles, m.p. 176° (gas evolution, evac. tube). $[\alpha]_D^{20}$ −649° (c = 1.0, $CHCl_3$).

Epimeric cis-8,14-dihydroxydihydrocodeines (VI). (*a*) *Via lithium aluminum hydride.* To a magnetically stirred mixture of 8.5 ml. (excess) of 1.65*N* lithium aluminum hydride and 35 ml. of dry ether was added, during 1.75 hr., a solution of 1 g. of V in 150 ml. of dry ether. The system was gently refluxed for 7 hr., then stirred (at 25°) for 15 hr. longer. After decomposing the excess reagent with water, 2*N* hydrochloric acid was added (to Congo acidity) and the suspension stirred into solution. The cooled aqueous phase was basified with a slight excess of 10*N* sodium hydroxide, nearly saturated with sodium chloride, and extracted three times with chloroform. Concentration (vacuum) of the dried extracts yielded a clear gum (0.9 g.) which crystallized when moistened with ethyl acetate and seeded with authentic *cis*-8,14-dihydroxydihydrocodeine (obtained from osmium tetroxide oxidation of o-acetylneopine).

The product was dissolved in boiling ethyl acetate, filtered, and concentrated to small volume; seeding induced the separation of colorless prisms (A). After 1.5 hr. the supernatant liquor was removed and the crystals rinsed with a little cold ethyl acetate (which was added to the mother liquor—see below). A second crystallization from ethyl acetate gave 0.46 g. of prisms, m.p. 177–179°. The analytical sample was recrystallized once again, m.p. unchanged.

Anal. Calcd. for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95. Found: C, 64.68; H, 6.84. $[\alpha]_D^{20}$ −144° (c = 1.0, 95% ethanol).

The infrared spectrum of this substance was indistinguishable from that of the osmium tetroxide–acetylneopine product; moreover no depression was observed in a mixture m.p.

Concentration (steam-bath) of the ethyl acetate mother liquors yielded a yellow gum which crystallized when rubbed with ether. Recrystallization from the latter solvent afforded 0.19 g. of flat, colorless prisms (B), m.p. 171–173°. A second recrystallization raised the m.p. to 174–176°. A mixture of this with epimer (A) showed the m.p. 158–160°; in addition, the respective infrared spectra differed significantly.

Anal. Calcd. for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95. Found: C, 64.80; H, 7.05. $[\alpha]_D^{20}$ −133° (c = 1.0, 95% ethanol).

The combined yield of epimers was 65%.

(*b*) *Via sodium borohydride.* A solution of 1.7 g. V in 17 ml. of absolute methanol was added dropwise (during 15 min.) to a suspension of 0.7 g. of sodium borohydride in 10 ml. of the same solvent. After stirring for 2.25 hr. longer, the system was acidified with 2*N* HCl and heated on the steam bath (reflux) for 10 min. Methanol was removed (*vacuum*). Water (10 ml.) and methanol (15 ml.) were added and the system concentrated (*vacuum*). The process was repeated once again. The aqueous residue was basified with saturated potassium carbonate and extracted six times with chloroform which yielded 0.8 g. of a foamy glass. This was triturated four times with 30 ml. portions of boiling ether; the combined extracts yielded 0.4 g. of colorless crystals, m.p. 160–168°. Soxhlet (ether) extraction of the residue (0.23 g.) during 60 hr. afforded 70 mg. of crystals, m.p. 160–164°, and 0.13 g. of insoluble material.

The aqueous solution from the chloroform extractions (above) was concentrated to dryness (steam bath) and the residue Soxhlet (chloroform) extracted during 15 hr. This yielded 0.8 g. of a yellow powder which was heated for 20 min. with a mixture of 20 ml. of methanol and 3 ml. of 2*N* HCl. After another methanol-acid treatment and the usual work-up (as above) the aqueous residue was basified with saturated potassium carbonate. The oily suspension crystallized when seeded with the above crystals (m.p. 160–168°). The air-dried product (0.7 g.) was recrystallized from ether (0.12 g. insoluble) and yielded 0.44 g. of faintly yellow prisms, m.p. 179–181°.

The combined ether-insoluble fractions (0.25 g.) were again treated with methanol and 2*N* HCl. This afforded an additional 95 mg. of crystals, m.p. 176–178°. The combined crystalline material (1.05 g.) represents a 60% yield of mixed epimers.

Separation of epimers by fractional recrystallization. The crystalline material was dissolved in boiling ethyl acetate, filtered, concentrated to small volume and seeded. After 36 hr. at 25°, the nearly colorless material was collected, 0.61 g. (crop I), m.p. 174–176°. Recrystallization from ether gave 0.58 g. of colorless prisms, m.p. 179–180.5°. A mixture of this substance with its counterpart (m.p. 177–179°) obtained *via* lithium aluminum hydride reduction (above) showed no m.p. depression.

From the further concentrated ethyl acetate mother liquor (after 24 hr. at 25°) there was obtained 0.19 g. (crop II) of crystals, m.p. 168–170°. Recrystallization from ether yielded 0.17 g. of flat prisms, m.p. 171–173°; not depressed when mixed with the corresponding lithium aluminum hydride fraction. A mixture of crops I and II showed the m.p. 161–163°.

The combined yield of recrystallized epimers was 48%.

True 14-Hydroxycodeine (VIII). A stirred suspension of 5 g. of VII[13] in 75 ml. of absolute methanol was gradually treated with 2 g. of sodium borohydride in 25 ml. of the same solvent. Stirring was maintained for 2 hr. and the solution was concentrated (vacuum) to one-half the original volume. After adding 50 ml. of 10% NaOH the solution was

boiled vigorously for a few moments and the remainder of the methanol removed (vacuum) whereupon the product crystallized. The latter was recrystallized from 50% ethanol; yield 4.3 g., m.p. 155–156°. A sample was sublimed at 150°/0.1 mm., m.p. 156–157°.

Anal. Calcd. for $C_{18}H_{21}NO_4$: C, 68.6; H, 6.71. Found: C, 68.5; H, 6.64. $[\alpha]_D^{20}$ −81.1° (c = 1, 10% HOAc).

14-Hydroxydihydrocodeine-B. A solution of 1 g. of *true* 14-hydroxycodeine in 30 ml. of 95% ethanol was shaken under hydrogen with 75 mg. of PtO_2. After the uptake of 1.2 moles of hydrogen (35 mins.), the usual manipulation yielded 0.84 g. of colorless crystals, m.p. 145–146°; the melting point was not depressed when mixed with 14-hydroxydihydrocodeine-B[13] (of m.p. 145–145.5°).

A small sample of the above product was acetylated (acetic anhydride–pyridine) and the product worked up as usual. Recrystallized from ethanol, the substance had the m.p. 181° alone or admixed with diacetyldihydrocodeine-B[13] (of m.p. 181–181.5°).

6,14-Diacetoxycodeine. A solution of 1.4 g. of *true* 14-hydroxycodeine in a mixture of 3 ml. of acetic anhydride and 1.5 ml. of dry pyridine was kept at 25° for 24 hr. The resulting crystalline magma was poured onto ice and treated slowly with 6*N* NH_4OH producing a colorless, crystalline precipitate which was collected and recrystallized from 95% ethanol; yield 1.2 g. colorless prisms. After a second recrystallization, m.p. 199° (evac. tube).

Anal. Calcd. for $C_{22}H_{25}NO_6$: C, 66.2; H, 6.31; CH_3CO, 21.5. Found: C, 66.1; H, 6.38; CH_3CO, 21.3. $[\alpha]_D^{20}$ −46.2° (c = 1.02, 10% HOAc).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents. V. Some Sulfur-Substituted Derivatives of Cysteine[1]

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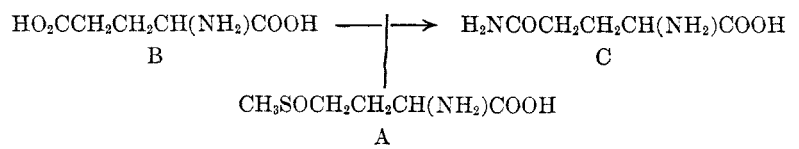
A number of *S*-alkyl and *S*-aryl derivatives of DL- and L-cystine, as well as some of their sulfoxides and sulfones, have been prepared for testing as possible anticancer agents.

In a search for compounds with anticancer activity, a number of derivatives of DL- and L-cysteine have been prepared. These compounds can be considered as potential amino acid antimetabolites; they could affect certain metabolic systems in a way similar to that in which methionine sulfoxide (A) acts as a glutamic acid (B) antagonist in the conversion of glutamic acid (B) to glutamine (C).[2]



The *S*-alkyl- and *S*-arylcysteines in Table I were prepared by a variety of methods. Direct alkylation of L-cysteine with reactive halogen compounds in the presence of dilute aqueous alkali and at room temperature gave good yields of compounds I–IV. The preparation of *S*-methyl-L-cysteine (V) was carried out with dimethyl sulfate and required a long reaction time in order to achieve a good yield. The *N*-benzoyl and *N*-acetyl derivatives of *S*-methyl-L-cysteine (V) were prepared by conventional procedures. The preparation of *S*-isopropyl-L-cysteine (VI) by direct alkylation of L-cysteine with isopropyl bromide or isopropyl iodide gave low yields of VI. The procedure of Gawron and Lieb[3] utilizing the alkylation with isopropyl bromide of the sodium salt of L-cysteine prepared from L-cystine in liquid ammonia gave a high yield of *S*-isopropyl-L-cysteine (VI).

The preparation of *S*-trimethylsilylmethyl-L-cysteine (VII) from L-cysteine and (chloromethyl)-trimethylsilane required a long reaction time in refluxing aqueous dioxane, an indication of the low order of activity of the halogen of the silane. An effort was made to prepare a phosphorylated compound by reaction of L-cysteine in alkali with (chloromethyl)phosphonic acid or dialkyl (chloromethyl)phosphonates. New ninhydrin-positive material was formed in these reactions as shown by paper chromatography, but efforts to purify the products were unsuccessful. Although many of the compounds in Table I are designated as derivative of L-cysteine, it is recognized that the conditions used in methods A, B, and D might lead to various degrees of racemization.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, and is in collaboration with the Sloan-Kettering Institute for Cancer Research. For the preceding paper of this series, *cf.* Elmer J. Reist, Leon Goodman, Roland R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 3962 (1958).

(2) E. Borek, P. Sheiness, and H. Waelsch, *Federation Proc.*, **5**, 123 (1946).

(3) O. Gawron and J. A. Lieb, *J. Am. Chem. Soc.*, **74**, 834 (1952).