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Note

Thin-layer and gas chromatographic parameters of morphine and codeine derivatives

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During the course of an investigation on the metabolism of morphine and codeine by *Arthrobacter* species No. 86 (ref. 1) several transformation products of these drugs were found.

Most of the work in the literature on the characterization of derivatives of morphine and codeine presume the previous chemical synthesis of those substances[2,3] since most of those derivatives are not commercially available. Availability of R_F data of these alkaloid derivatives in thin-layer chromatography (TLC) and gas chromatography (GC) would help in a rapid characterization, in many cases avoiding chemical synthesis of standard samples and studies of mass and infrared spectra of the metabolites. A very voluminous literature on separation of drugs of abuse by TLC and GC exists[4]. However, few data on separation of unusual derivatives of morphine and codeine are available.

Most of the products we have found as metabolites of morphine and codeine by bacteria[1] are more oxidized compounds. The only literature existing on oxidized metabolites of opium drugs are studies of the separation of fourteen hydroxycodeine derivatives by GC[5] and the separation of thebaine metabolites by instant TLC[3].

Data on TLC and GC of reduced compounds of the morphine and codeine series are present in several publications[6,7]. In this note we include TLC and GC data of some oxidized derivatives, that will contribute to the identification of bacterial metabolites of morphine and codeine.

MATERIALS AND METHODS

Morphine and codeine were purchased from Merck Sharp & Dohme, Rahway, N.J., U.S.A. The samples of other drugs used were a kind gift from Dr. E. L. May (NIH, Bethesda, Md., U.S.A.) and Dr. A. L. Misra (New York State Narcotic Addition Control Commission, New York, N.Y., U.S.A.). Silica gel G plates (Analtech Newark, Del., U.S.A.), 250 μm thick, 20 × 20 cm, scored in 1-cm-wide strips, were used for TLC. The plates were saturated before being developed in the corresponding solvent system at 24°. After development, plates were dried and the products were

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visualized with iodine vapor. GC was performed in a Bendix Model 2600 gas chromatograph. A 3 ft. × 1/4 in. glass U-shaped column, packed with 3 g of 3% OV-17 80–100 mesh Supelcoport 01-1953 (Supelco, Bellefonte, Pa., U.S.A.) was used. The operating conditions were: oven temperature, 210°; inlet temperature, 230°; detector temperature, 245°. The gases used were: helium as carrier gas at a flow-rate of 75 ml/min; hydrogen, 40 ml/min; and air, 380 ml/min. The drugs were dissolved in methanol at 1 mg/ml concentration, and 1-μl samples were injected. Morphine and codeine were used as internal standards.

RESULTS

A good separation of compounds derived from the same parent drug was achieved by GC (Table I), although individual compounds of both groups, such as 14-hydroxymorphine and 14-hydroxycodeinone, have similar retention times. Methylation of the hydroxyl group at C_3 produces a decrease in the retention time in GC of all the compounds of the codeine series in relation to the corresponding compounds of the morphine series, while an increase occurs in the mobility in TLC. Presence of a hydrox-

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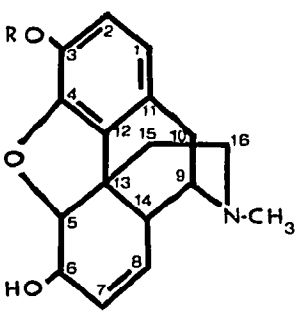
TENTION TIME DATA IN GAS CHROMATOGRAPHY AND MOBILITY IN THIN-LAYER CHRO-ATOGRAPHY OF DERIVATIVES OF OPIUM ALKALOIDS

= ethyl acetate–methanol–conc. ammonia (86:10:4); S_2 = chloroform–methanol (90:10); S_3 = ethanol–idin–dioxane–water (50:20:25:5); S_4 = ethanol–dioxane–benzene–conc. ammonia (10:40:50:10); S_5 = metha--butanol–benzene–water (60:15:10:15).



npound	*Structure*	*Relative retention time**	$R_F \times 100$				
			S_1	S_2	S_3	S_4	S_5
rphine	R=H	1.00	18	10.3	30.5	17	24
Hydroxymorphine	R=H, C_{14} β-OH	1.65	42	12	50.5	34	21
Hydroxymorphinone	R=H, C_{14} β-OH, C_6 =O	1.76	63	54	70	60	43
Hydroxydihydromorphinone	R=H, C_{14} β-OH, C_6 =O, Δ 7,8	1.45	63	43	62	64	27
ydromorphinone	R=H, C_6 =O, Δ 7,8	1.21	16	15	21	23	16
deine	R=CH_3	0.80	33	33	30.5	29	24
deinone	R=CH_3, C_6 =O	1.20	43	48	37	42	32
Hydroxycodeine	R=CH_3, C_{14} β-OH	1.32	65	45	55	66	28
Hydroxycodeinone	R=CH_3, C_{14} β-OH, C_6 =O	1.64	77	78	64	73	48
Hydroxydihydrocodeinone	R=CH_3, C_{14} β-OH, C_6 =O, Δ 7,8	1.45	80	70	52	81	28

* Relative to morphine (retention time = 7.32 min).

yl group at C_{14} and oxidation of the C_6 hydroxyl group produce lower mobilities in GC in both series, which agrees with results published for the codeine series[5]. An increase in mobility upon hydroxylation at C_{14} or oxidation at C_6 occurs in TLC, especially in alkaline solvents. Hydrogenation of the C_{7-8} double bond in morphine and codeine derivatives produces a decrease in the retention time in GC and lower mobility in most of the solvent systems in TLC. No differences in the R_F data of these compounds were found when the solvent system used was acid, such as 1-butanol–acetic acid–water (40:10:20) (not included in Table I). In summary, a good separation and an accurate identification of these metabolites is possible by the combined use of GC and TLC in one or more of the mentioned solvent systems.

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