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Note

Chemistry of opium alkaloids

VIII*. Separation of opium alkaloids and related compounds by ion-pair high-performance liquid chromatography

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In our synthetic investigations of the medicinally useful alkaloids morphine (1) and codeine (2) (Fig. 1) and related compounds[1–3,6] (Fig. 2), it was necessary also to separate closely related derivatives. The application of high-performance liquid chromatography (HPLC) to the analysis of morphinans has already been described[4,5], mainly with natural compounds. These methods were found to be unsuitable for the separation of non-substituted morphinans from, *e.g.*, synthetic[2,6] 1-methyl-substituted morphinans.

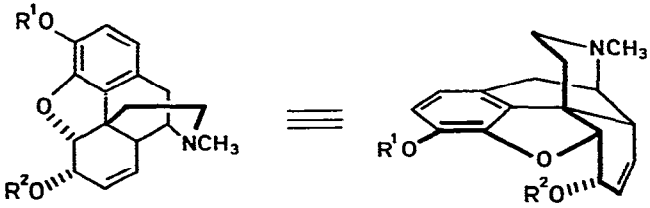

Fig. 1. Structure of alkaloids: $R^1 = R^2 = H$, morphine (1); $R^1 = CH_3$, $R^2 = H$, codeine (2); $R^1 = R^2 = CH_3CO$, heroin (3).

When use is made of ion-pair chromatography[7] with *n*-heptanesulphonate as the counter ion over a reversed-phase column, these analyses can be carried out satisfactorily. The analysis of natural morphinans has also been considerably improved by this means.

EXPERIMENTAL

Materials

Compounds 1–6, 11, 20 and 21 were obtained from the Verenigde Pharmaceutische Fabrieken, B.V., Apeldoorn, The Netherlands, while compounds 7, 8, 15

* For Part VII, see ref. 3.

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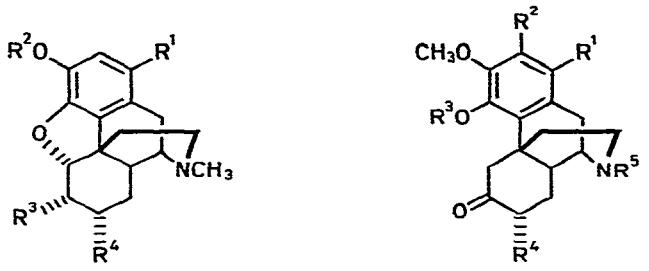


Fig. 2. Structures of morphinan derivatives analysed by HPLC:

No.	R^1	R^2	R^3	R^4	*No.*	R^1	R^2	R^3	R^4	R^5
4	H	H	OH	H	11	H	H	H	H	CH_3
5	H	CH_3	OH	H	12	H	H	$COCH_3$	H	CH_3
6	H	CH_3	=O	H	13	H	H	H	H	H
7	CH_3	CH_3	OH	H	14	H	H	H	H	CHO
8	CH_3	CH_3	=O	H	15	CH_3	H	H	H	CH_3
9	Br	CH_3	=O	H	16	CH_3	H	H	H	CHO
10	Br	CH_3	=O	Br	17	Br	H	H	H	CH_3
20	Thebaine				18	Br	H	H	Br	CH_3
21	Oripavine				19	H	OH	H	H	CH_3

and 16; 9, 10, 17 and 18; 12, 13 and 14; and 19 were synthesized according to refs. 2, 6, 8, 3 and 1, respectively.

Apparatus

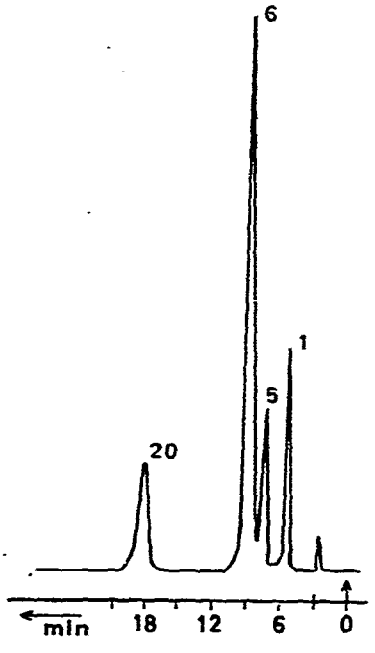
A Waters Assoc. Model 6000 A pump with a Model U6K injector was used in combination with a Varian Aerograph UV detector at 254 nm. The column was a μBondapak C_{18} from Waters Assoc. The flow-rate was set at 1.2 ml/min and the column was maintained at room temperature. The mobile phase contained 0.005 *M* *n*-heptanesulphonic acid (PIC reagent B-7) obtained from Waters Assoc. (Milford, Mass., U.S.A.).

RESULTS AND DISCUSSION

The analysis of morphinans on a silica gel column has already been described[4,5]. The most important opium alkaloids that have been separated by this method are morphine, codeine, heroin, thebain, 6-(O-acetyl)morphine, dihydromorphine, dihydrocodeine and nalorphine, the structures and basicity of which are closely related.

We have repeated the analysis of a number of these alkaloids on μPorasil, with chloroform–methanol–diethylamine, diethyl ether–methanol–diethylamine and methanol–2 *N* ammonia–1 *N* ammonium nitrate in water. For alkaloids with high retention times the results were not satisfactory, especially owing to tailing of the peaks. Application of this technique to a number of closely related morphinans and to the derivatives of dihydrothebainone did not yield good separations, while some of them were retained on the column. The use of a reversed-phase column (μBondapak C_{18}) in combination with methanol–0.1 *N* ammonium hydrogen carbonate also resulted in bad separations and much tailing of the peaks.

Ion-pair chromatography on a reversed-phase column gave very good separations of some important morphinan alkaloids (Fig. 3). From Fig. 4, it can be seen that this technique gives good results for the separation of closely related morphinans, *e.g.*, 1-methyldihydrocodeine from dihydrocodeine.



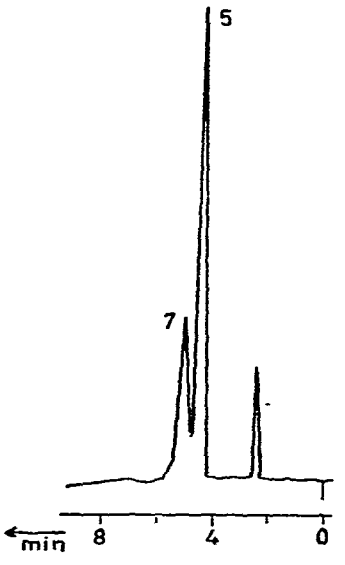


Fig. 3. Separation of some important morphine alkaloids. Morphine (1), dihydrocodeine (5), dihydrocodeinone (6), and thebaine (20) were separated on μBondapak C_{18} using methanol–water (40:60) containing 0.005 *M* *n*-heptanesulphonic acid. Flow-rate, 1.2 ml/min; UV detection at 254 nm.

Fig. 4. Chromatogram of dihydrocodeine (5) and 1-methyldihydrocodeine (7) on μBondapak C_{18} using methanol–water (50:50) containing 0.005 *M* *n*-heptanesulphonic acid. Flow-rate, 1.2 ml/min; UV detection at 254 nm.

The retention times increase as the polarity of the molecule decreases (Table I). By varying the methanol (or acetonitrile) to water ratio in the mobile phase, the retention times can be varied, an increase in the water content resulting in an increase in the retention time. With the aid of gradient elution (increase in methanol concentration), various alkaloids can be separated in a reasonably short time. The compounds can be injected in the form of a salt (*e.g.*, hydrochloride) or as the free base, with no change in the retention times. Non-basic compounds, such as the N-formyl compounds, can also be separated under these circumstances. It is striking that the N-formyl compounds give two partially overlapping peaks (14 and 16), which is attributed to the *syn–anti* isomerism of the N-formyl group. Rotation around the formyl nitrogen–carbon bond is sufficiently slow for this purpose at room temperature.

For the separation of many other alkaloids and basic compounds, the reversed-phase, ion-pair mode of HPLC appears to offer good prospects.

TABLE I

RETENTION TIMES (min) OF MORPHINANS FOR DIFFERENT SOLVENT SYSTEMS (CONTAINING 0.005 *M* *n*-HEPTANESULPHONIC ACID)

Dashes indicate no elution within a reasonable time (> 30 min).

Compound	*Solvent system*		
	Methanol–water (50:50)	*Methanol–water (40:60)*	*Acetonitrile–water (25:75)*
1	4.0	5.6	4.5
2	4.6	7.5	6.1
3	7.3	19.4	16.2
4	4.1	6.0	4.9
5	4.5	7.7	5.7
6	5.1	8.8	8.0
7	5.4	9.6	7.6
8	5.9	12.7	11.5
9	8.0	18.7	17.6
10	14.5	—	—
11	5.2	9.0	7.5
12	5.5	10.1	8.7
13	5.1	9.5	6.7
14	5.1, 5.3	9.2, 9.8	10.0
15	6.0	12.6	10.1
16	6.5, 7.0	13.3, 14.7	14.4
17	8.0	21.4	14.0
18	11.7	—	—
19	4.0	5.7	5.6
20	7.5	17.9	17.5
21	4.8	8.7	7.5

ACKNOWLEDGEMENT

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