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Note

High-performance liquid chromatographic separation of alkaloids from *Papaver somniferum* on a Zorbax NH₂ analytical column*

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The levels of alkaloids in opium gum and related preparations have been determined by normal^{1,2}, reversed-phase³, and ion-exchange⁴ high-performance liquid chromatographic (HPLC) procedures. Numerous alkaloids are present in capsule tissues of *Papaver somniferum*, including papaverine, thebaine, narceine, codeine, and morphine. Recovery studies have been carried out^{1,3} to optimize the extraction of these compounds from capsule latex.

In this report, we demonstrate that HPLC on a Zorbax*** NH₂ analytical column, used in the weak anion-exchange mode, allows an improved and rapid separation of the compounds listed above. Also, tyrosine is shown to be a good internal standard for quantitation, being well separated from the other compounds and having an adequate ultraviolet absorption at 286 nm. The potential of this mode of chromatography for the efficient separation of mixtures of hydrophilic weak acids was demonstrated in earlier studies with ascorbic acid and closely related compounds⁵.

EXPERIMENTAL

Reagents

Thebaine, codeine, and morphine were obtained as gifts (Penick Corporation, NJ, U.S.A.), and papaverine and narceine were purchased from Applied Science Labs. (State College, PA, U.S.A.).

Instrumentation

The modular HPLC system consisted of an Instrumentation Specialties Co. (ISCO, Omaha, NE, U.S.A.) metering pump 314, series 1240-003, a pressure monitor (ISCO Model 1590), a Perkin-Elmer (Norwalk, CT, U.S.A.) LC-55 variable-wavelength UV detector, a DuPont (Wilmington, DE, U.S.A.) Zorbax NH₂ analytical column (250 × 4.6 mm I.D.), and an Alltech Scientific (Deerfield, IL, U.S.A.) 20- μ l

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*** Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

loop injector. A Hewlett-Packard 3390A reporting integrator was used for recording and quantitation.

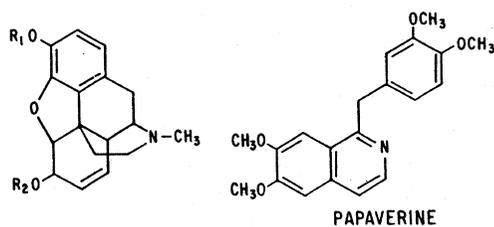
Extraction and separation procedures

Freeze-dried latex from *P. somniferum* was extracted using the procedures described by Vincent and Engelké¹, and also the procedure of Wu and Wittick³.

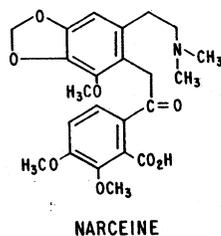
Optimal separations were achieved by use of the Zorbax NH₂ column in the weak anion-exchange mode, with a mobile phase consisting of acetonitrile-0.025 M KH₂PO₄ (75:25 v/v). The standard mixture, consisting of the title compounds, was effectively separated at a flow-rate of 2.0 ml/min, with the internal standard tyrosine eluting after morphine. The flow-rate of eluent was changed to 1.0 ml/min when analyzing latex extracts, since interfering peaks complicated the chromatogram at the higher flow-rate. Both the standard mixture and the extracts were analyzed as solutions in the mobile phase, with 85% H₃PO₄ added dropwise to permit dissolution of all components. The injected standard mixture consisted of the following levels of each alkaloid in 20 μg of mobile phase: papaverine, 0.75 μg; thebaine, 0.75 μg; narceine, 0.97 μg; codeine, 5.32 μg; morphine, 6.60 μg. Tyrosine, the internal standard, was present at a level of 10.69 μg. A wavelength of 286 nm was used to detect all compounds of interest.

RESULTS AND DISCUSSION

The structures of the compounds of interest in the present study are given in Fig. 1. Both codeine and morphine are derived biosynthetically from thebaine by stepwise enzymatic demethylations. Fig. 2 shows the HPLC separation of these compounds and the internal standard tyrosine, and Table I summarizes the chromatographic behavior of these compounds. The column is being used in the weak anion-exchange mode, so only those compounds with ionizable protons are strongly re-



MORPHINE R₁ and R₂ = H
CODEINE R₁ = CH₃, R₂ = H
THEBAINE R₁ and R₂ = CH₃



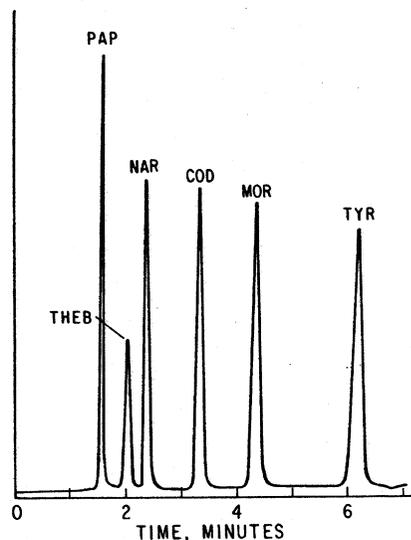


Fig. 2. Separation of standard mixture; papaverine (PAP), thebaine (THEB), narceine (NAR), codeine (COD), and morphine (MOR). Chromatographic conditions are described in Experimental section.

tained. Papaverine and thebaine have no anionic character, so they are eluted rapidly; reversed-phase³ HPLC is better equipped to quantitate these compounds, as they are the last eluted in this series. We found noscapine (another *Papaver* alkaloid) to co-elute with papaverine, but these are well separated by reversed-phase HPLC.

The procedure described here is especially useful for the rapid determination of narceine, codeine, and morphine. These compounds have capacity ratios (k') much greater than unity. In reversed-phase HPLC, these compounds are eluted near the column void volume and possess low k' values. At a flow-rate of 2.0 ml/min, all standard compounds and tyrosine are eluted in little more than 6 min, and these conditions should suffice for relatively simple mixtures, such as some pharmaceutical preparations. Extracts from the latex of *P. somniferum* however, are quite complex and include several compounds with UV absorbance at 286 nm. We suggest therefore, that the flow-rate be reduced from 2.0 to 1.0 ml/min for the analysis of these extracts.

TABLE I
HPLC EVALUATION OF SEPARATE COMPOUNDS ON ZORBAX NH₂

Compound	Retention time (min)	k'	α^*	Response _{tyrosine}
Papaverine	1.62	0.157	0.046	5.37
Thebaine	2.03	0.450	0.132	6.82
Narceine	2.42	0.728	0.213	6.14
Codeine	3.32	1.371	0.402	1.093
Morphine	4.24	2.029	0.595	0.906
Tyrosine	6.18	3.41	1.000	1.000

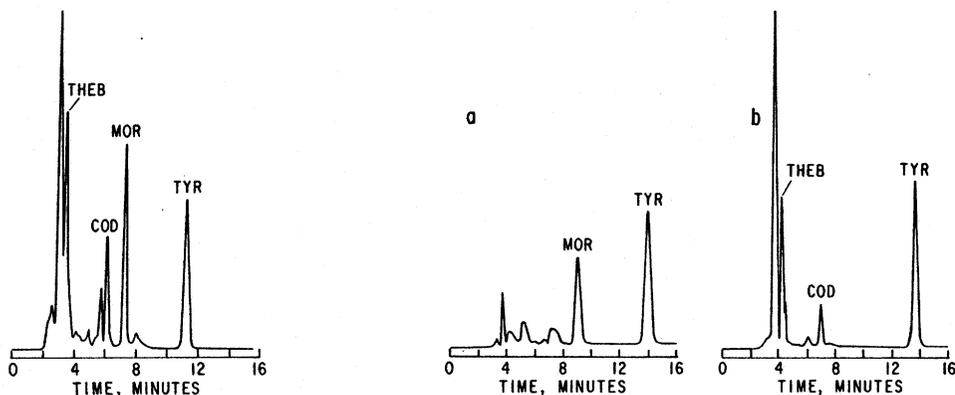


Fig. 3. Chromatogram of mixture extracted¹ from latex of *Papaver somniferum*. Chromatographic conditions are described in Experimental section.

Fig. 4. Chromatogram of mixture extracted³ from latex of *Papaver somniferum*. (a) Morphine-containing fraction with internal standard tyrosine; (b) thebaine- and codeine-containing fraction with internal standard tyrosine. Chromatographic conditions are described in the Experimental section.

This allows the chromatogram to be spread, minimizing the overlapping of peaks which interfere with the peaks of interest.

Figs. 3 and 4 represent chromatograms of samples of lyophilized latex from capsules of *P. somniferum*, extracted according to the procedures of Vincent and Engelké¹ and Wu and Wittick³, respectively. In Fig. 3, the alkaloids of interest (thebaine, codeine, and morphine) are revealed in the chromatogram, while in Fig. 4, two chromatograms are required, since thebaine and codeine were extracted from the initial aqueous phase with methylene chloride. In the present study, the dried extracts of both procedures were dissolved in mobile phase containing the internal standard tyrosine. Both procedures are quite suitable for the determination of codeine and morphine; but the chromatogram in Fig. 4 is best for thebaine, since thebaine is better separated from the earlier eluting mixture near the column void volume.

The weak anion-exchange chromatographic mode described here very efficiently resolves the alkaloids of interest, representing a significant improvement over existing procedures. Particular advantage may be derived when using the procedure for the rapid determination of these compounds in pharmaceutical preparations and synthetic mixtures.

REFERENCES

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