Journal of Chromatography, 315 (1984) 404–411
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,170

Note

Analysis of opium and crude morphine samples by capillary gas chromatography

Comparison of impurity profiles

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(Received August 23rd, 1984)

Opium, in terms of amounts seized, is the second most abused drug, cannabis being the first. In 1981 the amount seized was ten times that of heroin[1]. The characterization of opium and crude morphine is also important, because these are the precursors for heroin processing.

Up to now, packed column gas chromatography (GC) has mainly been used for the study of the main constituents of opium[2–11] and crude morphine[12], either underivatized[2–6] or derivatized[7–12]. Because we have successfully developed and applied several capillary GC methods for the characterization of heroin samples[13–17] and especially studied different new heroin impurities with these methods[13,14,16,17], we became interested in detailed chromatographic profiles of the precursors.

This paper reports the application of high-resolution capillary GC to the analysis of opium and crude morphine samples. A simple method for the direct identification and determination of the main and minor constituents after silylation without prior extraction[10] or column separation[11,18] is presented. Preliminary results concerning the profiling of trace impurities[16,17] in opium and crude morphine samples after extraction from the bulk drug are reported. A first comparison of these impurity profiles with those obtained from processed heroin is discussed.

EXPERIMENTAL

Apparatus

All chromatography was performed on a Varian Vista 6000/6500 GC system with flame-ionization detection (FID) and thermionic specific detection (TSD, N-FID) systems and interfaced with a Varian Vista CDS 401 data system. The gas chromatograph was fitted with an all-glass Gerstel-Splitter (Mülheim, F.R.G.), a make-up gas device and 25 m × 0.27 mm I.D. cross-linked glass capillary columns coated with OV-1 or SE-54 (film thickness 0.15 μm). Hydrogen was used as the carrier gas at a flow-rate of *ca.* 110 cm/sec (OV-1) or 65 cm/sec (SE-54) and measured at an oven temperature of 150°C. Argon was used as the make-up gas at a flow-rate of 18 ml/min. Chromatograms were recorded at an attenuation of $128 \cdot 10^{-12}$ (OV-1) or $64 \cdot 10^{-12}$ A/mV (SE-54) and a chart speed of 1.0 cm/min.

0021-9673/84/$03.00

Chemicals and samples

Pyridine and toluene were obtained from Merck (Darmstadt, F.R.G.) and chloroform of Chrom AR quality from Promochem (Wesel, F.R.G.). MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) was obtained in 10-ml vials from Macherey, Nagel & Co. (Düren, F.R.G.). All other chemicals were of analytical-reagent grade quality.

The standard opium alkaloids and acetyl derivatives were used as the pure bases. The internal standards tetracosane and dotetracontane were supplied by Merck and Applied Science (Oud-Beijerland, The Netherlands), respectively.

Eleven crude morphine samples and nine opium samples [two of the latter from Merck "Opium eingestellt (10% Morphin nach DAB 7" and "Opium gepulvert (12% Morphin nach DAB 7)"] were used.

Procedures

Quantitative determination. For the quantitation of morphine, codeine, thebaine, papaverine and narcotine (noscapine) and the detection of other constituents, about 5 mg of crude morphine or 10 mg of opium were accurately weighed together with 1 mg tetracosane as internal standard. The mixture was subjected to silylation with 150 μl of MSTFA in 1.2 ml of chloroform–pyridine (5:1, v/v) for 10 min at 70°C according to the previously published method for heroin analysis[15]. After 1 h at room temperature for crude morphine or overnight for opium samples, 1 μl was injected. An OV-1 capillary column was used with the injector at 250°C, detector at 280°C and an oven temperature programme from 150 to 280°C at a rate of 9°C/min and then 0.5 min isothermal.

Profiling. The recently published method for heroin impurity profiling[16,17] was slightly modified for opium and crude morphine. The sample was 15 mg of opium (finely ground) or 80 mg of crude morphine and was dissolved in 5 ml of 0.5 *M* H_2SO_4 by shaking for 1–4 min. For effective extraction of the acidic solution in 1 min, 5 ml of toluene (containing 6 μg/ml of *n*-dotetracontane as internal standard) were added and shaking was effected with a Vibrofix VF 1 shaking device (IKA Janke & Kunkel, Staufen, F.R.G.). The mixture was then centrifuged for 10 min and the toluene layer was separated and evaporated to dryness in a conical centrifuge tube. To the residue 50 μl of toluene and 40 μl of MSTFA were added and the solution was heated at 70°C for 5 min. About 1 μl was injected into the GC system, which was equipped with an SE-54 capillary column. The injector was maintained at 270°C and the detector at 300°C; an oven temperature programme from 150 to 280°C at a rate of 6°C/min, 1 min isothermal at 280°C, then at 15°C/min to the final temperature of 300°C with a final hold of 20 min was used. The profiling of opium required another hold of the GC oven for 30 min at 300°C.

Acetylation. A 5-g amount of crude morphine and 50 ml of acetic anhydride were refluxed for 2 h and, after cooling to room temperature, the acetic anhydride was evaporated, the residue dissolved in 150 ml of water, the solution filtered and the product precipitated with concentrated Na_2CO_3 solution (25%). The crude base was filtered, washed with 100 ml of water and dried for 1 h at 105°C. It was stored in a desiccator over P_2O_5.

RESULTS AND DISCUSSION

Capillary GC analysis of main and minor constituents

As was expected, high-resolution capillary GC proved to be a very valuable tool for the characterization of opium and crude morphine samples. Especially with opium samples the complex mixture of opium alkaloids, fatty acids, sugars and other plant substances is easily separated after trimethylsilylation (Fig. 1A) and well resolved chromatograms for forensic comparisons are generated. The chromatogram of a typical opoium sample (opium bread) from the German illicit market, shown in Fig. 1A, is characterized by many minor constituents in addition to morphine, narcotine, papaverine, codeine and thebaine. The enhanced efficiency of the capillary GC method is demonstrated by comparison with previously published packed column studies[7,10].

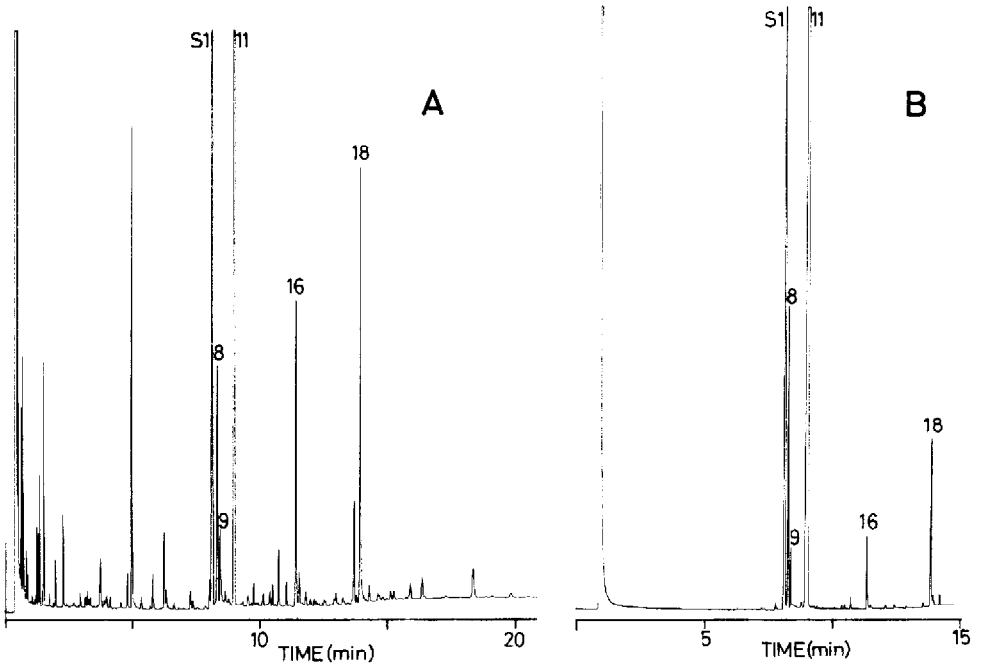

Fig. 1. Capillary gas chromatograms of an opium sample (A) and a crude morphine sample (B). For peak identification, see Fig. 2.

The chromatogram of an illicit crude morphine base from Southwest Asia (Fig. 1B), in comparison with opium, shows a much cleaner product and again a good separation of the opium alkaloids after trimethylsilylation. Exact quantitation of thebaine using GC can be a problem owing to decomposition of the compound under the conditions normally employed for the analysis[19]. Our sensitive capillary GC method illustrated this problem by larger amounts of thebaine showing a poor peak shape and tailing.

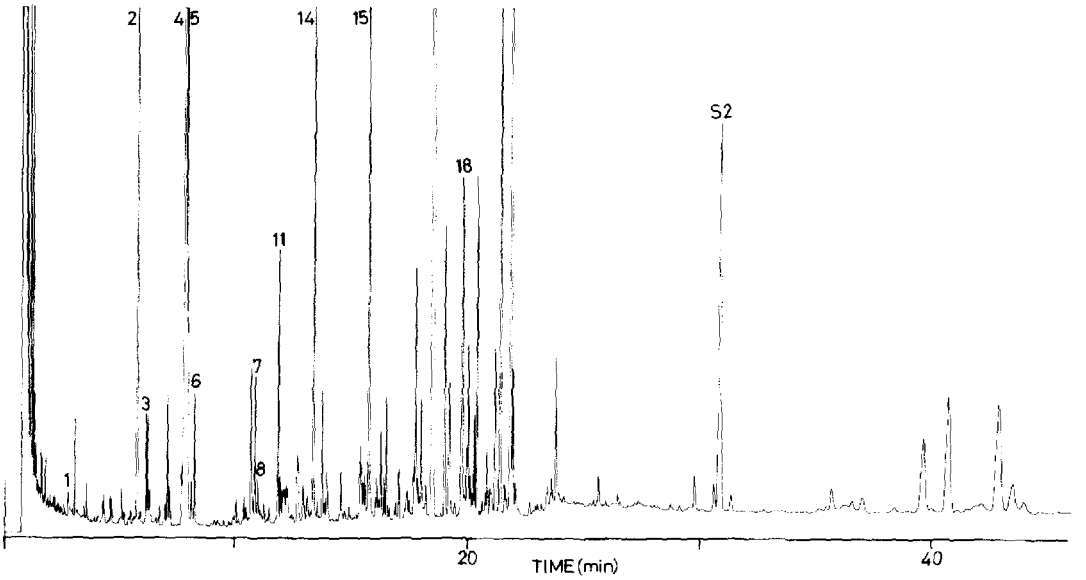

Fig. 2. Capillary GC impurity profile of opium with FID. Peaks: 1 = meconin; 2 = palmitic acid-TMS; 3 = palmitic acid; 4 = linoleic acid-TMS; 5 = oleic acid-TMS; 6 = stearic acid-TMS; 7 = thebaol-TMS; 8 = codeine-TMS; 9 = thebaine; 11 = morphine-TMS; 14 = behenic acid-TMS; 15 = lignoceric acid-TMS; 16 = papaverine; 17 = papaveraldine; 18 = narcotine; S1 = tetracosane (internal standard); S2 = dotetracontane (internal standard) (TMS = trimethylsilyl derivative).

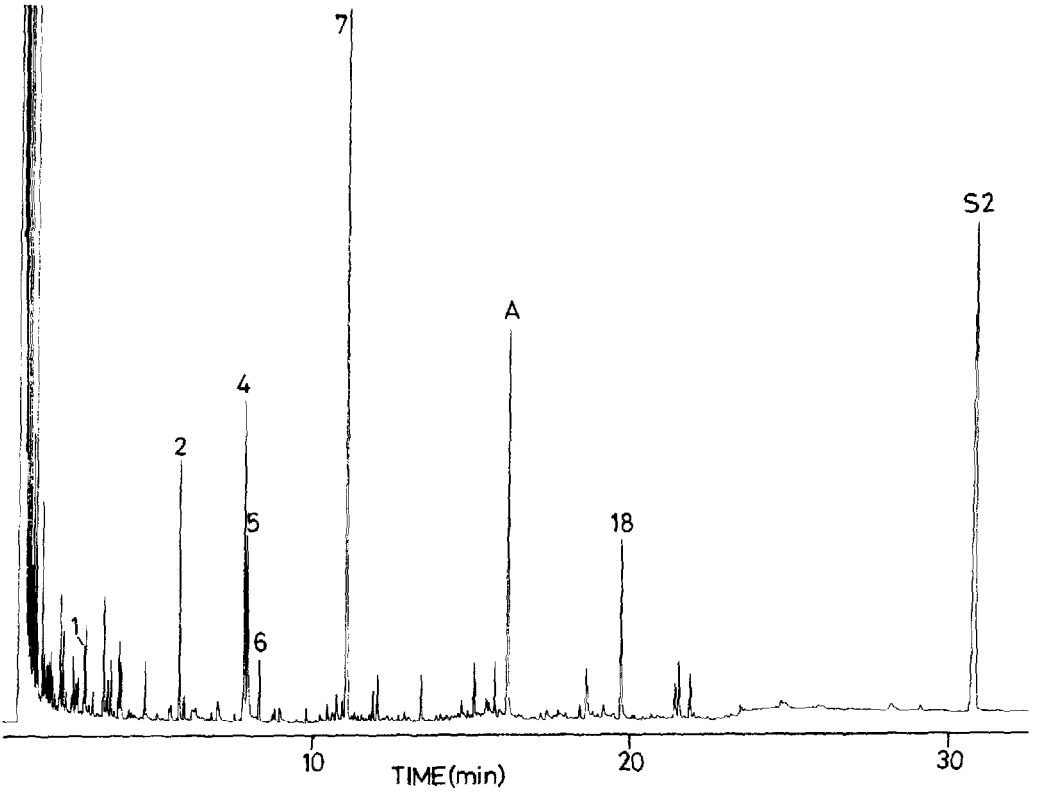

Fig. 3. Capillary GC impurity profile of crude morphine sample I (*cf.*, Fig. 1B) with FID (peak A, containing N, not amenable to trimethylsilylation). For peak identification, see Fig. 2.

Profiling of trace impurities

With some slight modifications, the capillary GC procedure developed for profiling of illicit heroin impurities after extraction from the bulk drug matrix was also applicable to opium and crude morphine samples.

A representative capillary GC impurity profile of opium (Fig. 2) with FID after trimethylsilylation shows a good separation of the very complex mixture. About 150 peaks were recorded during the time of 45 min shown in Fig. 2, and more compounds are also eluted later. Investigation of the profile with nitrogen-sensitive FID shows that only a small number of the components contain nitrogen, morphine (peak 11) being the most abundant. Most of the constituents representing the profile in Fig. 2 must therefore be attributed to non-alkaloid substances. Hence most of the

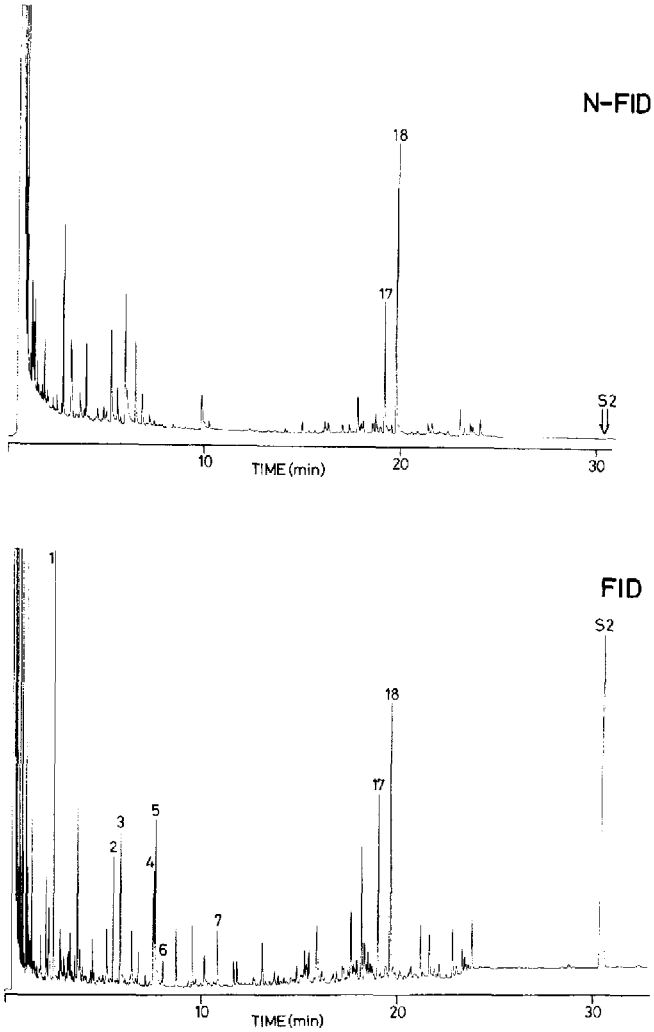

Fig. 4. Capillary GC impurity profiles of crude morphine sample II with FID and N-FID. For peak identification, see Fig. 2.

compounds identified so far are fatty acids. With respect to this class of organics, studies of the underivatized impurity extracts by capillary GC were not very promising.

For two different crude morphine samples, the chromatograms shown in Figs. 3 and 4 were generated using the profiling procedure. Again derivatization was essential in order to obtain a complex chromatographic profile. Comparison of detection with FID and nitrogen-sensitive FID (N-FID) for the same sample in Fig. 4 demonstrates that FID is more suitable for the type of samples investigated here. The profiles of crude morphine were characterized mostly by fatty acids, narcotine, thebaol and meconin.

Preliminary comparison of impurity profiles of opium, crude morphine and illicit heroin

A first comparison of the impurity profiles of illicit heroin[16,17] with that of crude morphine and opium showed much greater similarities between opium and crude morphine impurities. This is understandable because only solution, extraction and precipitation procedures are used for the preparation of the crude morphine[12]. The acetylation used to produce heroin, however, changes the impurity pattern drastically. As reported previously[17], most compounds representing the heroin impurity profiles are due to synthetic by-products of the opium alkaloids morphine, codeine,

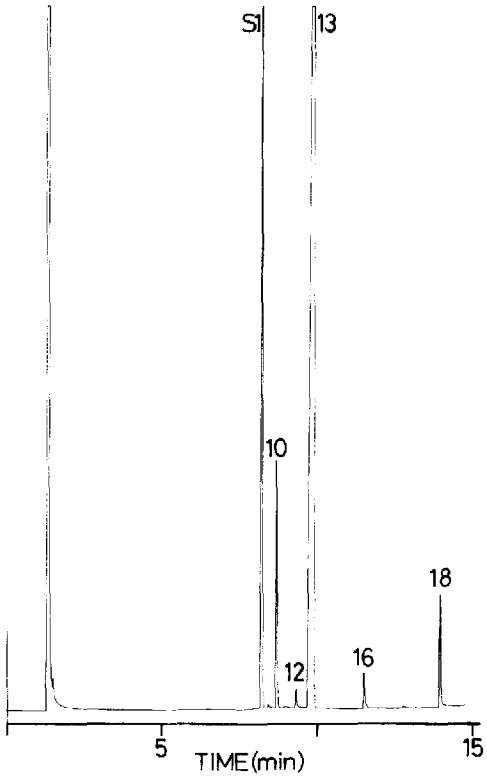

Fig. 5. Capillary gas chromatogram of the acetylation product of crude morphine sample I. Peaks: 10 = acetylcodeine; 12 = 3-O-TMS-6-O-acetylmorphine; 13 = diacetylmorphine; 16 = papaverine; 18 = narcotine; S1 = tetracosane (internal standard).

thebaine, narcotine and norlandanosine generated during the acetylation process.

In a brief investigation of the effects of acetylation on the impurities, the crude morphine base (Figs. 1B and 3) was treated with acetic anhydride. The main constituents formed are shown in Fig. 5 and the impurity profile of the acetylation product is illustrated in Fig. 6. The latter chromatographic pattern can easily be classified as a typical Near/Middle East heroin profile. Repeated preparations yielded a good comparable heroin impurity profile. It is very different from the impurity profile of the precursor morphine (Fig. 3), thus making correlations based on chromatographic profiles in this respect difficult. A better approach for comparing illicit heroin with the precursor morphine would be the use of selected ratios of constituents or derivatives[20] present in both substances.

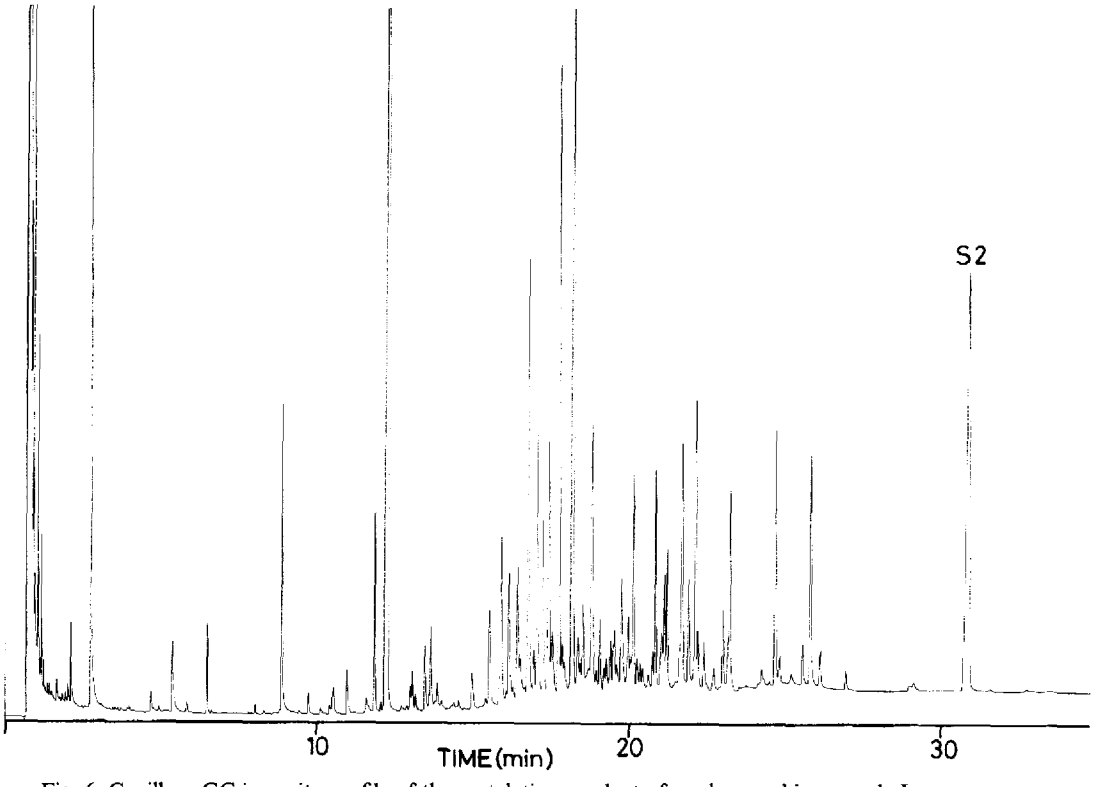

Fig. 6. Capillary GC impurity profile of the acetylation product of crude morphine sample I.

CONCLUSIONS

A convenient, rapid and efficient capillary GC method for the direct determination of opium alkaloids in opium and crude morphine has been presented. It has been demonstrated that highly specific chromatograms of impurities in these substances can be obtained by using capillary GC for profile analysis after extraction from the bulk drug. A preliminary comparison of these impurity profiles with that

from illicit heroin revealed much greater similarities between opium and crude morphine than between opium and heroin.

ACKNOWLEDGEMENT

The author thanks Hans-Peter Meyer for excellent technical assistance.

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