Henk Huizer,[1] *Ph.D.*

Analytical Studies on Illicit Heroin
I. The Occurrence of O^3-Monoacetylmorphine

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ABSTRACT: A thin-layer chromatographic method and a high pressure liquid chromatographic method have been developed for the analysis of O^3-monoacetylmorphine in illicit heroin samples. The possible formation of O^3-monoacetylmorphine during the production process of heroin and during its hydrolysis were also studied using these methods.

KEYWORDS: toxicology, chromatographic analysis, O^3-monoacetylmorphine, heroin

Samples of illicit heroin usually contain varying amounts of O^6-monoacetylmorphine (6-MAM) (Fig. 1). Several methods have been described for the detection and the quantitative determination of this compound in heroin samples. In addition to thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) [*1-4*] also high pressure liquid chromatography (HPLC) has increasingly been used during the last few years [*2,5-8*]. Whereas the content of 6-MAM in illicit heroin samples has been found to vary from 1 to 43% [*2,9,10*], little information on the occurrence of O^3-monoacetylmorphine (3-MAM) (Fig. 1) is available.

TLC on Alumina E has been used to study the stability of aqueous heroin solutions [*11,12*]. However, the TLC systems used in those studies do not permit detection of small amounts of 3-MAM present in illicit heroin samples. Better results have been achieved by TLC on silica gel [*1*], whereby heroin and both monoacetylmorphines could be separated and detected. However, the system was found unsuitable for Dutch illicit heroin samples because of the occurrence of other compounds in the samples, which interfered with the detection of 3-MAM: caffeine in samples originating from the Far East and a not identified compound in samples from the Middle East.

GLC with an electron capture detector has been performed with 3-MAM after derivatization with heptafluorobutyric acid anhydride [*13*]. Amounts of 3-MAM varying from 0.1 to 2% were found in uncut heroin samples. Although detection limits were not given, other investigations have indicated that a limit of 0.01% might be expected [*14*].

In many papers published on the analysis of heroin samples by means of HPLC [*2,5-8, 15-18*] nothing has been mentioned about 3-MAM. However, in an investigation on the stability of heroin injections, an HPLC separation of morphine, both monoacetylmor-

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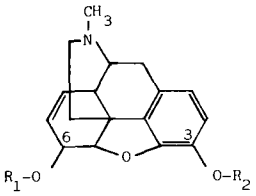
[1]Chemist and head of Drug Investigation Section, Gerechtelijk Laboratorium, Rijswijk, The Netherlands.



O^3- monoacetylmorphine: R_1=H, R_2=$COCH_3$

O^6- monoacetylmorphine: R_1=$COCH_3$, R_2=H

FIG. 1—*Chemical structure of monoacetylmorphines.*

phines and heroin, was described [*19*]; retention data for compounds, possibly present in illegal heroin samples, were not given.

Hays et al [*20*] reported an HPLC separation of morphine, 6-MAM, 3-MAM, codeine, acetylcodeine, and heroin. Because 3-MAM was eluted immediately after 6-MAM and because 6-MAM always occurred in much greater amounts than 3-MAM in the samples analyzed during our studies, no satisfactory determination of small amounts of 3-MAM might be expected.

There is quite a difference of opinion as to the origin of the monoacetylmorphines present in heroin samples. The occurrence of 6-MAM was attributed to hydrolysis of heroin during salt formation and to storage conditions of heroin [*9,10,19,21-23*]. Davey and Murray [*24*] reported that 3-MAM was sometimes formed by hydrolysis, but the main decomposition of heroin proceeded via 6-MAM. Some workers were of opinion that 6-MAM originated from an incomplete acetylation of morphine [*23,25*]. Moore and Klein [*13*] found, however, that an incomplete acetylation did not lead to 6-MAM, but to 3-MAM; 6-MAM was mainly a product formed by hydrolysis of heroin.

In the present paper we describe a TLC and an HPLC procedure by means of which the occurrence of 3-MAM in illicit heroin samples can be established, even when other alkaloids are present, namely, acetylcodeine, codeine, caffeine, morphine, 6-MAM, noscapine, papaverine, and strychnine. By means of these procedures the formation of the monoacetylmorphines during acetylation of morphine and during hydrolysis of heroin can also be studied.

Experimental Procedure

Materials

Thirty-two illicit heroin samples, seized on the Dutch black market, were used. Sixteen of them consisted of the so-called Far Eastern type and the other sixteen consisted of the so-called Middle Eastern type. Commercial samples of heroin and morphine hydrochlorides were used as references. O^6-monoacetylmorphine hydrochloride was obtained as a gift from the firm Diosynth (Apeldoorn, The Netherlands). O^3-monoacetylmorphine was prepared according to Welsh [*26*], and its purity was checked by means of TLC and HPLC; only traces of heroin were detected.

Apparatus and Methods

Thin-Layer Chromatography—Precoated plates (Silica gel 60 GF 254, Merck, Darmstadt, West Germany) were used. The mobile phase consisted of toluene-acetone-ethanol-diethylamine (30:60:7:3).

Detection was achieved by heating for 3 h at 110°C; bright blue fluorescent spots of the morphine esters were observed in 366-nm ultraviolet light.

High Pressure Liquid Chromatography—A liquid chromatograph SP 8000 (Spectra

Physics, San Jose, CA) equipped with an ultraviolet detector (227-nm wavelength) and a stainless steel column (250 mm long and 4.6 mm inner diameter) filled with Lichrosorb Si-60-7 (Merck) was used. The mobile phase consisted of hexane-dichloromethane-methanol (75:20:5); the methanol contained 0.75% (v/v) diethylamine. The flow rate was 1.5 mL/min.

Sample Preparation—With TLC, 30 mg of the heroin samples was dissolved in 1.0 mL of methanol; 10 μL of the solution was applied.

With HPLC, 30 mg of the heroin samples was dissolved in water, gradually with the addition of tartaric acid. The solution was saturated with sodium bicarbonate and extracted twice with dichloromethane. The solvent was evaporated on a water bath and the residue dissolved in 10 mL of the solvent mixture used as mobile phase. A 100-μL sample was injected into the chromatograph via a loop injection system. Injection of a heroin sample as salt gave a negative peak with a retention time about the same as that of 3-MAM.

Formation of Monoacetylmorphines—In order to study the acetylation of morphine during a commonly used acetylation process 5 g of morphine hydrochloride was boiled with 150 mL of acetic acid anhydride. At regular intervals small samples were applied on TLC plates and the excess of acetic acid anhydride removed in a cold air stream. For HPLC a 0.5-mL sample was neutralized with 8 mL of 1*N* sodium hydroxide and 1 g of sodium bicarbonate. This solution was extracted twice with dichloromethane. Further preparation proceeded as described for the sample preparation with HPLC.

Results and Discussion

Thin-Layer Chromatography

The mobile phase used by Stahl [*27*] for the separation of opium alkaloids (toluene-acetone-ethanol-ammonia 25% [45:45:7:3]) was modified, since it was observed that the morphine esters hydrolyzed during TLC because of the ammonia, resulting in tailing spots. For this reason the ammonia was replaced by diethylamine. The R_f values of the compounds are summarized in Table 1.

The amount of 3-MAM in the samples was usually too small for detection by spraying with potassium iodoplatinate. A more sensitive and more specific detection was obtained by heating the plates for 3 h at 110°C. Thereby bright blue fluorescent spots visible in ultraviolet light (366 nm) were formed. Detection limit for 3-MAM was 0.06 μg (equals about 0.02% of the sample analyzed).

High Pressure Liquid Chromatography

A typical chromatogram of a heroin sample originating from the Far East and containing 0.3% 3-MAM is given in Fig. 2. The retention volumes of compounds possibly present in illicit heroin samples are listed in Table 2. 3-MAM was well separated from the other compounds—and by means of the technique described about 0.05% could be detected and quantitatively determined by using the external standard method. A linear relationship between the peak area and the amount of substance was observed.

Content of Monoacetylmorphines

By means of TLC 3-MAM was detected in almost all heroin samples investigated. Samples originating from the Middle East contained usually less than 0.1%. For quantitation of such small amounts HPLC was less suitable. The heroin content in these samples varied from 65 to 80%.

In heroin samples originating from the Far East 3-MAM was found in amounts between

TABLE 1—R_f *values of some constituents found in illicit heroin samples. Mobile phase: toluene-acetone-ethanol-diethylamine (30:60:7:3).*

Morphine	0.15
Strychnine	0.30
Codeine	0.33
O^3-monoacetylmorphine	0.37
O^6-monoacetylmorphine	0.41
Acetylcodeine	0.45
Heroin	0.46
Caffeine	0.58
Procaine	0.70
Papaverine	0.70
Noscapine	0.89
Meconine	0.89

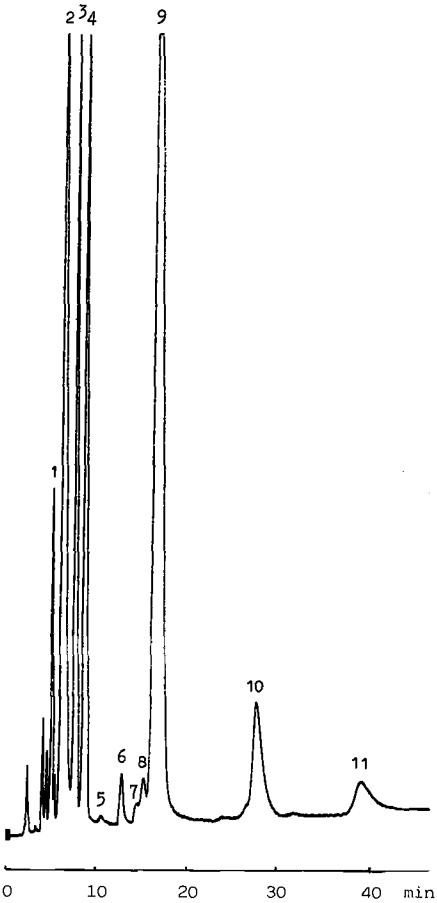


FIG. 2—*A typical chromatogram of a heroin sample originating from the Far East and containing 0.3% 3-MAM where* 1 = *barbital,* 2 = *caffeine,* 3 = *acetylcodeine,* 4 = *heroin,* 5 = *unknown,* 6 = O^3-*monoacetylmorphine,* 7 = *unknown,* 8 = *codeine,* 9 = O^6-*monoacetylmorphine,* 10 = *strychnine, and* 11 = *morphine.*

TABLE 2—*Retention volumes (mL) of some constituents found in illicit heroin samples. Column: Lichrosorb Si-60-7. Mobile Phase: hexane-dichloromethane-methanol (75: 20: 5); the methanol contained 0.75% (v/v) diethylamine.*

Noscapine	5.9
Barbital	7.2
Papaverine	7.9
Caffeine	9.1
Acetylcodeine	11.2
Heroin	12.4
Procaine	13.9
O^3-monoacetylmorphine	19.6
Codeine	23.7
O^6-monoacetylmorphine	25.7
Strychnine	42.2
Morphine	58.4

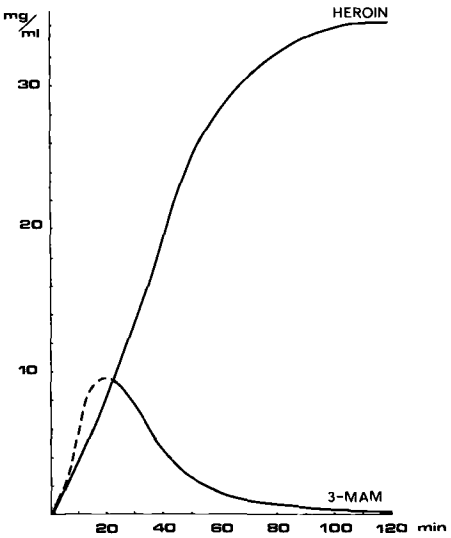


FIG. 3—*Concentrations of heroin and 3-MAM versus time during the acetylation process.*

0.1 and 0.5% by HPLC; 0.3% was most common. In these samples the heroin content was about 30%.

Considerable differences were observed for the amounts of 6-MAM in the samples originating from the Middle East and the Far East. The Middle Eastern samples contained 1 to 2% 6-MAM, whereas the samples from the Far East usually contained 10%—sometimes as much as 17%.

Formation of Monoacetylmorphines

TLC showed that during the acetylation of morphine (see Experimental Procedure) 3-MAM was immediately formed, and then heroin. By means of HPLC the results given in

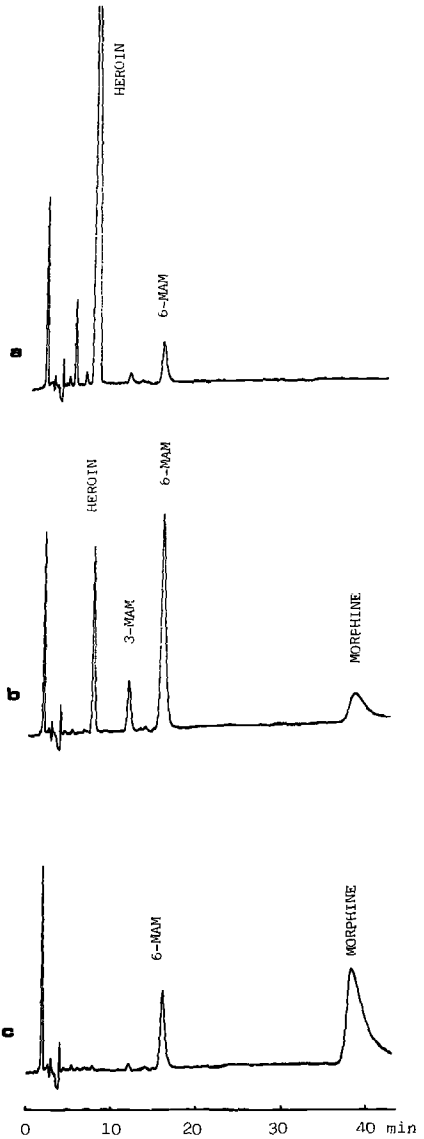


FIG. 4—*Formation of monoacetylmorphines from heroin in a solution of 30% hydrochloric acid, as established by HPLC where* a = *after 1 min,* b = *after 15 min, and* c = *after 60 min.*

Fig. 3 were obtained. The formation of 3-MAM during the first 10 min was not included, since TLC showed that morphine still was present in the reaction mixture. This morphine might give 3-MAM during the preparation of the sample for HPLC.

It is known that illegal heroin manufacturers normally use smaller amounts of acetic acid anhydride [*28*] than those used for our studies. Under those conditions the amounts of 3-MAM may be expected to be larger.

During the acetylation no detectable amounts of 6-MAM were formed. This is in agreement with the results of Moore and Klein [*13*].

TABLE 3—*The stability of O^3-monoacetylmorphine in 30% hydrochloric acid and 25% sodium carbonate.*

	Concentration 3-MAM (mg/mL)	
t (min)	in HCl	in Na_2CO_3
0	3	3
1	3	2.8
15	2	1.3
30	0.8	0.6

The low content of 3-MAM in heroin samples might also be attributed to its decomposition during the following steps in the heroin preparation process: first it is exposed to an excess of sodium carbonate, next it comes in contact with strong hydrochloric acid during the preparation of the hydrochloride of heroin. The stability of 3-MAM was investigated by adding it to 30% hydrochloric acid and to an aqueous solution of 25% sodium carbonate, respectively. The results are given in Table 3.

The formation of monoacetylmorphines from heroin was studied by adding heroin hydrochloride to 30% hydrochloric acid and to an aqueous solution of 25% sodium carbonate, respectively. According to the literature, first 6-MAM is formed rapidly in both cases and then morphine. By TLC and HPLC it was established that also 3-MAM was formed (Fig. 4) in a maximum amount after about 10 min, whereafter its amount decreased and the amount of morphine increased.

Conclusion

The illicit heroin samples originating from the Far East showed a high content of 6-MAM and a low content of 3-MAM. The high 6-MAM content is probably caused by an unskilled preparation of the heroin hydrochloride. The occurrence of 3-MAM might be caused either by an incomplete acetylation or by hydrolysis of the heroin.

The heroin samples from the Middle East, which are characterized by extremely small amounts of both monoacetylmorphines, are obviously prepared by skilled manufacturers.

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Address requests for reprints or additional information to
Dr. H. Huizer
Gerechtelijk Laboratorium Van Het Ministerie Van Justitie
Volmerlaan 17
2288 GD Rijswijk, The Netherlands