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DETERMINATION OF NALOXONE AND NALTREXONE AS PERFLUOROALKYL ESTER DERIVATIVES BY ELECTRON-CAPTURE GAS–LIQUID CHROMATOGRAPHY

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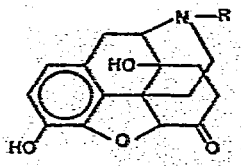
SUMMARY

An electron-capture gas chromatographic method is described for the determination of naloxone and naltrexone as the perfluoroalkyl esters. Each compound serves as internal standard for determination of the other. The method permits quantitation of 2–100 ng of either compound. Conditions for derivatization with heptafluorobutyric anhydride (HFBA), pentafluoropropionic anhydride (PFPA), and trifluoroacetic anhydride (TFAA) have been investigated. When catalyzed with pyridine, derivatization with HFBA and PFPA at 70° gives naloxone and naltrexone triesters. Evidence for triester formation was obtained from gas chromatography–methane chemical ionization mass spectrometry and infrared spectral analysis. It was found that both the HFB and PFP triesters are suitable for quantitation of the narcotic antagonists, the HFB derivatives having greater stability than the PFP derivatives. The TFA derivatives are substantially less stable.

INTRODUCTION

Naloxone (Ia) and naltrexone (Ib) are potent, rapidly acting narcotic antagonists currently undergoing extensive clinical trials for prevention of narcotic addiction in man[1–3].



Ia: R = $CH_2CH{=}CH_2$

Ib: R = CH_2— 

They are active at low plasma concentrations, typically in the ng/ml range[4], and are extensively metabolized[5–12]. Thus, analytical methodology of high sensitivity

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and specificity is required to study the mechanism of action as well as the absorption, distribution, and elimination of these compounds.

Verebely *et al.*[13] have recently reported a gas chromatographic assay for naltrexone and its metabolite, β-naltrexol in human urine which is based upon flame ionization detection (FID) of the silylated derivatives. They reported that the absolute sensitivity of detection of these compounds is 10–20 ng/ml. On examining the plasma concentration–time profiles following intravenous administration of naltrexone in dogs and monkeys, it was found that the data is best fit to a two-compartment open model[14]. Plasma levels in the terminal phase of the profiles are in the range 2–20 ng/ml following a 0.72 mg/kg dose to a dog and in the range 10–45 ng/ml following a 1-mg/kg dose to a monkey. These concentrations are in the region of the absolute sensitivity of the FID method. Accordingly, a method having greater sensitivity is required for a pharmacokinetic study utilizing plasma samples.

This report describes conditions for submicrogram scale derivatization of naloxone and naltrexone with perfluoroalkyl anhydrides and for determination of the resulting derivatives by electron-capture gas–liquid chromatography (GLC–ECD).

EXPERIMENTAL

Reagents

Naloxone hydrochloride (Ia-HCl) and naltrexone hydrochloride (Ib-HCl) were obtained from the National Institute on Drug Abuse (Rockville, Md., U.S.A.) and were converted to free bases. Purity was established by thin-layer chromatography (TLC) and by GLC–ECD of the heptafluorobutyryl derivatives.

Trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), heptafluorobutyric anhydride (HFBA), and heptafluorobutyryl imidazole (HFBI) were obtained from Pierce (Rockford, Ill., U.S.A.) and were used without further purification. They were carefully protected from moisture and were stored under refrigeration. Pyridine of Sequanal® quality was also obtained from Pierce and was stored under nitrogen at −5°.

Methanol and benzene of "Nanograde" purity were purchased from Mallinckrodt (St. Louis, Mo., U.S.A.). All other chemicals were reagent grade or better and were used as purchased.

Solutions containing 1% pyridine in benzene for use as a catalyst were prepared fresh daily.

Glassware

All glassware was soaked overnight in sulfuric acid–nitric acid (4:1). After thorough rinsing in distilled water, it was siliconized by immersion for one min in a 1% solution of Siliclad® (Clay Adams, Parsippany, N.J., U.S.A.) in water. Siliclad solutions were adjusted to pH 8–9 by the addition of concentrated ammonium hydroxide and were prepared fresh. Glassware was immediately rinsed with 50% methanol in water, and then dried at 105°.

Stock solutions

Stock solutions of Ia and Ib in methanol (0.01 mg/ml) were prepared. No evidence of decomposition was observed after storage of the solutions for six months at −5°.

Submicrogram derivatization of naloxone and naltrexone

Samples containing submicrogram quantities of naloxone and naltrexone in 15 × 125-mm glass culture tubes (Pyrex® No. 9826) were prepared from stock solutions of the compounds in methanol. Solvent was carefully evaporated under a stream of nitrogen. To each tube were added 25 μl of anhydride and 50 μl of 1% pyridine in benzene. Contents were thoroughly mixed and the tubes were tightly sealed with Teflon®-lined screw caps. Tubes were then placed in an oil bath (pre-heated to 70°) to a depth of about 50 mm so that the upper parts of the tubes acted as reflux condensers. After 2 h the tubes were placed in an ice bath.

Excess derivatizing agent was removed before chromatography by adding 5 ml of a saturated aqueous solution of sodium borate to the cooled reaction mixture. Tubes were immediately rotated on a Labquake® mixer for 3.0 min and then centrifuged for 4.0 min to separate the layers. When a series of samples was derivatized, excess agent was removed from each sample just before it was chromatographed in order to minimize hydrolysis of derivatives. Aliquots (1–3 μl) of the upper (organic) layers were immediately withdrawn into a 10 μl syringe and injected on to the GC column.

Electron-capture gas–liquid chromatography

Analyses were made using a Hewlett-Packard 5700A gas chromatograph equipped with a ^{63}Ni electron-capture detector (pulsed, variable-frequency type). Chromatographic columns were 183 cm × 2 mm I.D., coiled, borosilicate glass treated with trimethylchlorosilane (5% solution in toluene). With the aid of gentle vacuum, columns were packed with either 3% OV-1 or 3% OV-17 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.). Packed columns were conditioned at 325° for 4 h with no carrier gas flow, and then at 275° for 16 h with a carrier gas flow-rate of about 10 ml/min. Columns conditioned in this manner were ready for use without further treatment.

The carrier gas was argon–methane (95:5) at a flow-rate of 40 ml/min. For separations on OV-1 columns, the oven was programmed from 190° to 270° at 4°/min. Separations on OV-17 columns were carried out isothermally at 205°. The injection port and detector temperatures were 250°.

Gas chromatography–chemical ionization mass spectrometry

Low resolution mass spectra were obtained at Battelle Columbus Laboratories (Columbus, Ohio, U.S.A.) on a computerized GLC–mass spectrometry (MS) system consisting of a Varian 1740 gas chromatograph coupled to a Finnigan quadrupole mass spectrometer. Naloxone and naltrexone derivatives were chromatographed on a 183 cm × 2 mm I.D., glass column packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q. The column oven was programmed from an initial temperature of 190° to 270° at 4°/min. The injection port and detector temperatures were 280° and 290°. The carrier gas, methane (flow-rate, 20 ml/min), also served as reactant gas in the ionization chamber.

Derivatives were prepared by reacting 1 μg of naloxone or naltrexone with 50 μl of HFBA and 100 μl of 1% pyridine in benzene at 70° for 20 min. Excess reagent was removed by washing the reaction mixtures with saturated sodium borate solution as described earlier.

Plots of total ion current *versus* scan number were obtained and compared with chromatograms obtained by GLC–ECD on 3% OV-1. Mass spectra of major peaks were recorded.

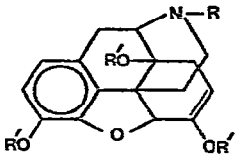
Infrared analysis

Infrared (IR) spectra of derivatives in benzene solution were obtained on a Perkin-Elmer 257 grating IR spectrometer using 0.1-mm sodium chloride cells.

Derivatives for IR analysis were prepared by heating 10 mg of naloxone with 50 μl of HFBA and (a) 500 μl of benzene or (b) 500 μl of 1% pyridine in benzene at 70°. Reaction mixtures were sampled periodically by removing 50-μl aliquots. After decomposition of excess HFBA and dilution with benzene, the aliquots were examined by GLC–ECD on the OV-17 column. When chromatograms indicated the presence of one derivative (either IIa or IIIa), the reaction mixtures were washed with sodium borate solution and centrifuged to separate the layers. The supernatant organic layers were removed and dried with anhydrous sodium sulfate before their IR spectra were obtained.

RESULTS AND DISCUSSION

Reaction of submicrogram quantities of naloxone and naltrexone with HFBA in the presence of pyridine results in derivatization of the hydroxyl groups at C-3 and C-14 as well as the carbonyl group at C-6 to yield the tri-substituted heptafluorobutyryl derivatives, IIa and IIb. Structures of IIa and IIb were confirmed by GLC–MS and IR spectroscopy.



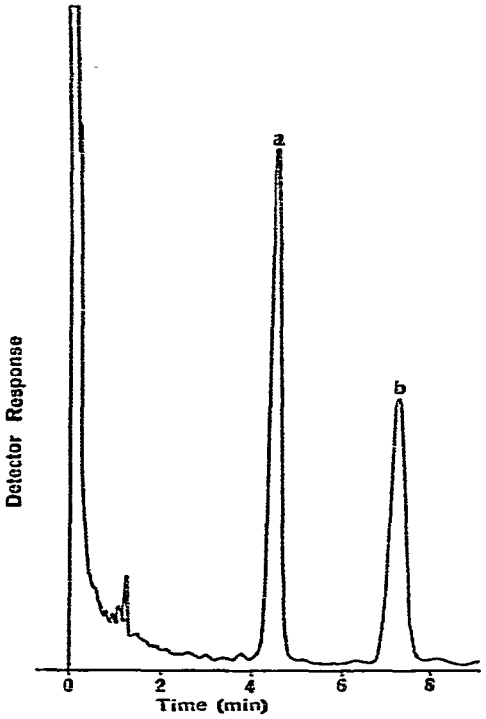
IIa: R = $CH_2CH{=}CH_2$

IIb: R = CH_2— 

R' = $\overset{O}{\overset{\|}{C}}CF_2CF_2CF_3$

The derivatives appear as single, symmetrical peaks by GLC–ECD (Fig. 1) and are suitable for quantitation. Minimum detectable quantities correspond to about 40 pg of Ia and 70 pg of Ib injected on-column.

For quantitative studies, naloxone and naltrexone were used as internal standards for each other. Because of the differences in the ECD responses to IIa and IIb, 100 ng of naltrexone was used for naloxone (10–100 ng) determinations and 60 ng of naloxone was used for naltrexone (10–100 ng) determinations. Standard curves were obtained by plotting peak height ratios against the amounts of naloxone or naltrexone added to standard samples. Fig. 2 shows a typical standard curve for the determination of 10–100 ng of naloxone with 100 ng of naltrexone added as internal standard. All standard curves were linear with zero intercepts and were very reproducible. Precision was about 1.0% relative standard deviation at 100 ng of naloxone ($n = 8$) and 8.5% at 10 ng ($n = 5$). High ECD responses of derivatives and high precision of peak height ratios permit accurate quantitation of 2–100 ng of either naloxone or naltrexone.



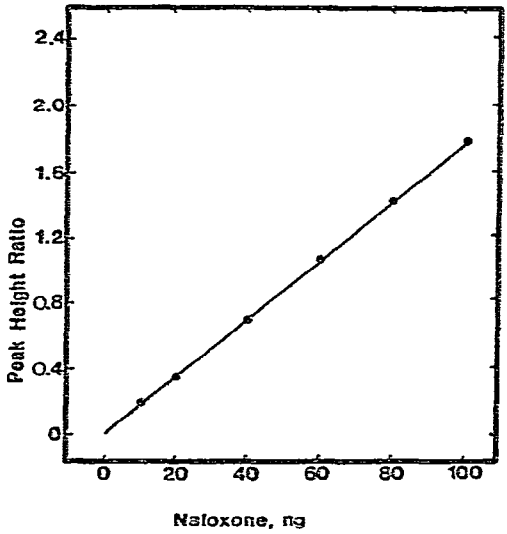


Fig. 1. Representative chromatogram of heptafluorobutyrate derivatives of (a) naloxone (100 ng) and (b) naltrexone (100 ng). Derivatization with HFBA catalyzed with pyridine. Column, glass (6 ft. × 2 mm), packed with 3% OV-17 on Gas-Chrom Q; column temperature, 205°; carrier gas, 5% methane in argon at a flow-rate of 40 ml/min.

Fig. 2. Calibration plot of 10–100 ng of naloxone assayed as its 3,6,14-HFB derivative. Internal standard, naltrexone-3,6,14-HFB.

Reaction conditions for submicrogram derivatization of naloxone and naltrexone to the corresponding tri-substituted heptafluorobutyryl derivatives, IIa and IIb, were investigated. The rate of formation of IIa was determined under the conditions described in Experimental with the exception that 9-bromophenanthrene was used as the internal standard instead of naltrexone. Reaction mixtures were heated at 70° for 3, 6, 9, 12, 15, 30, 60 and 120 min and then were quenched by decomposing the HFBA with sodium borate solution. The plot of peak height ratios (IIa/9-bromophenanthrene) against derivatization time (Fig. 3) indicates that derivatization is complete in about 1 h. The peak height ratio observed after heating for 16 h was identical with the ratio observed after heating for 1 h.

When pyridine was omitted from the HFBA derivatizations of naloxone and naltrexone, the major derivatives which were obtained exhibited longer retention times and lower ECD responses (Tables I and II) than the corresponding tri-substituted derivatives, IIa and IIb. Small quantities of IIa and IIb were also detected by GLC–ECD thus suggesting that derivatization is incomplete without the addition of pyridine.

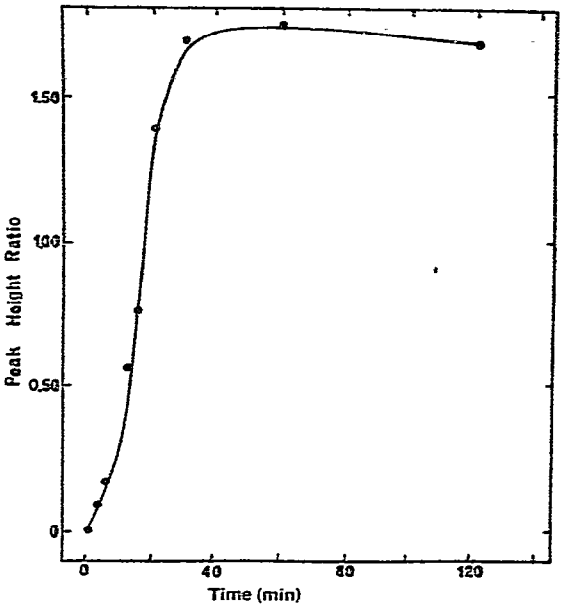


Fig. 3. Rate of formation of naloxone-3,6,14-HFB from 100 ng of naloxone and 25 μl HFBA in 50 μl benzene catalyzed by 1% pyridine at 70°.

A preliminary GLC–MS study indicated that the derivatives (IIIa and IIIb) result from esterification of the hydroxyl groups at C-3 and C-14.

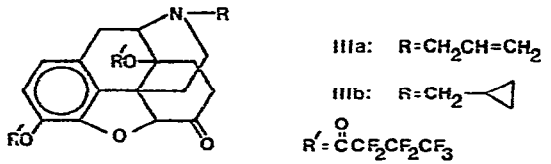


Fig. 4 shows a methane chemical ionization mass chromatogram of a mixture of naloxone and naltrexone derivatized with HFBA using pyridine catalyst and con-

TABLE I

RETENTION TIMES OF NALOXONE AND NALTREXONE DERIVATIVES RELATIVE TO 9-BROMOPHENANTHRENE

Separations were carried out on 3% OV-17 on Gas-Chrom Q at 205° with carrier gas flowing at 40 ml/min.

Compound	*Relative retention time*					
	Derivatization with pyridine			*Derivatization without pyridine*		
	TFAA	*PFPA*	*HFBA*	*TFAA*	*PFPA*	*HFBA*
Naloxone	0.898	0.658	0.680	0.898	1.45	1.46
Naltrexone	1.55	1.12	1.15	1.55	2.49	2.46

TABLE II

PEAK HEIGHTS AND PEAK AREAS OF NALOXONE AND NALTREXONE DERIVATIVES RELATIVE TO 9-BROMOPHENANTHRENE

Separations were carried out on 3% OV-17 on Gas-Chrom Q at 205° with carrier gas flowing at 40 ml/min.

Compound	*Peak height (peak area)*					
	Derivatization with pyridine			*Derivatization without pyridine*		
	TFAA	*PFPA*	*HFBA*	*TFAA*	*PFPA*	*HFBA*
Naloxone	2.89 (2.69)	2.59 (2.29)	2.81 (2.37)	2.89 (2.69)	1.42 (2.02)	1.03 (1.60)
Naltrexone	1.35 (1.95)	1.56 (1.87)	1.64 (2.03)	1.35 (1.95)	0.575 (1.43)	0.487 (1.14)

ditions so that partial derivatization of the molecules occurs. Mass spectra were obtained at the centroids of each of the peaks and background spectra were subtracted. The peaks in these spectra are listed in Table III. Peak 1 was found to correspond to naloxone-3,6,14-HFB. The only prominent ions in the mass spectrum have masses of 215, 197 and 169. These ions correspond to $[CF_3CF_2CF_2COOH \cdot H]^+$, $[CF_3CF_2CF_2CO]^+$, and $[CF_3CF_2CF_2]^+$, respectively. Since neither the molecular ion nor any fragments containing naloxone were observed, the structure could not be established from the MS data. However, confirmation of the structure was obtained by IR analysis. The mass spectrum obtained at peak 2 corresponds to naloxone-3,14-HFB. The spectrum shows a protonated molecular ion, $[M \cdot H]^+$, at 720 and significant fragment ions at 534, 506, 310, 215, 197, and 169. The ions at 534 and 506 result from loss of $CF_3CF_2CF_2COOH$ from $[M \cdot C_2H_5]^+$ and $[M \cdot H]^+$, respectively. The ion at 310 is due to loss of $[CF_3CF_2CF_2CO]^+$ from the ion at 506 to give a neutral molecule with a mass of 309. Protonation of this molecule gives an ion of mass 310. The ions at 215, 197, and 169 result from fragmentations similar to those observed with the naloxone triester. The mass spectrum of naltrexone-3,6,14-HFB was obtained at the centroid of peak 3. The spectrum exhibits ions of masses 732, 716, 520, 215, and 197. Although no molecular ion is observed, an ion of mass 716 corresponding to fragmentation of $CF_3CF_2CF_2COOH$ from the protonated molecular ion is observed thereby suggesting the identity of the compound. The ion of mass 520 results from loss of $[CF_3CF_2CF_2CO]^+$ from the ion of mass 716 to give a neutral molecule of mass 519. Protonation of this molecule results in an ion of mass 520. The ion of mass 732 is presently unexplained. The spectrum obtained at peak 4 shows that the peak corresponds to a mixture of two compounds, naloxone-3-HFB and naltrexone-3,14-HFB. The only ion which could be positively attributed to naloxone-3-HFB is the protonated molecular ion of mass 524. Ions in the spectrum having masses 734, 548, 520, 324, 215, and 169 result from the fragmentation of naltrexone-3,14-HFB. The protonated molecular ion at mass 734 confirms the identity of the compound. The ions of mass 548 and 520 originate from fragmentation of $CF_3CF_2CF_2COOH$ from $[M \cdot C_2H_5]^+$ and $[M \cdot H]^+$, respectively. Peak 5 is due to underivatized naloxone. The spectrum is characterized by a protonated molecular ion of mass 328. Peak 6 in the mass chroma-

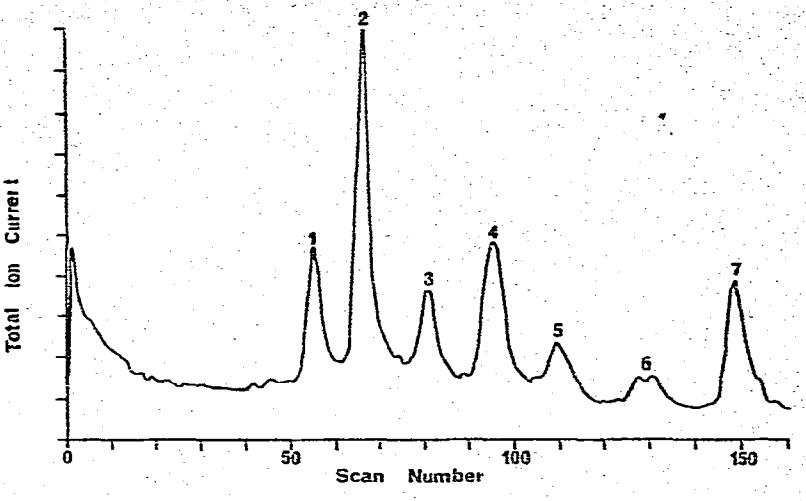


Fig. 4. Mass chromatogram of HFBA derivatives of naloxone and naltrexone: 1 = naloxone-3,6,14-HFB, 2 = naloxone-3,14-HFB, 3 = naltrexone-3,6,14-HFB, 4 = naltrexone-3,14-HFB and naloxone-3-HFB, 5 = naloxone, 6 = naltrexone-3-HFB, and 7 = naltrexone.

togram is due to naltrexone-3-HFB. The only significant ions in the mass spectrum of the monoester are found at masses 566, 538, and 342. Ions of masses 566 and 538 correspond to $[M \cdot C_2H_5]^+$ and $[M \cdot H]^+$, respectively, whereas the ion of mass 342 results from loss of $[CF_3CF_2CF_2CO]^+$ from the protonated molecular ion followed by protonation of the neutral molecule. Because the phenolic esters fragment with loss of $[RCO]^+$ whereas alcohols fragment with loss of $[RCOOH]^+$[15], the spectrum is attributed to naltrexone-3-HFB. Peak 7 is due to underivatized naltrexone. The spectrum exhibits ions of masses 370 and 342 corresponding to $[M \cdot C_2H_5]^+$ and $[M \cdot H]^+$, respectively.

Evidence that enol ester formation has not occurred at C-6 in the diesters IIIa and IIIb was obtained by comparison of the IR spectra of the diesters and triesters.

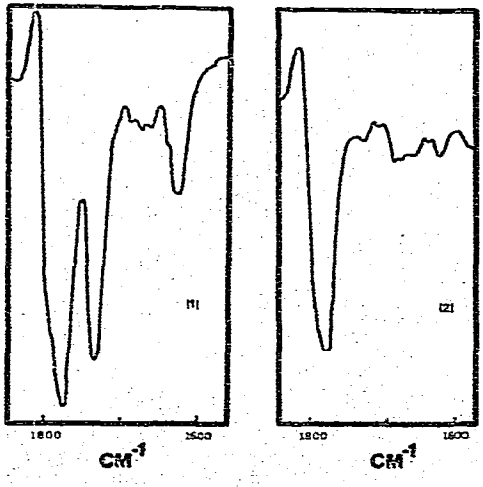


Fig. 5. Partial IR spectra of (1) naloxone-3,14-HFB (IIIa) and (2) naloxone-3,6,14-HFB (IIa).

TABLE III

METHANE CHEMICAL IONIZATION MASS SPECTRAL DATA OF THE MAJOR CHROMATOGRAPHIC PEAKS (FIG. 4) ON PARTIAL DERIVATIZATION OF NALOXONE AND NALTREXONE WITH HFBA

Peak	*m/e*	*Assignment**	*M Fragment*
1	215	$RCOOH \cdot H^+$	
	197	$RCOOH \cdot H^+ - H_2O$	
	169	R^+	
2	720	$M \cdot H^+$	M = Naloxone-3,14-HFB
	534	$M \cdot C_2H_5^+ - RCOOH$	
	506	$M \cdot H^+ - RCOOH$	
	310	$M \cdot H^+ - RCOOH - RCO^+ + H^+$	
	215	$RCOOH \cdot H^+$	
	197	$RCOOH \cdot H^+ - H_2O (= RCO^+)$	
	169	R^+	
3	732	Unknown	M = Naltrexone-3,6,14-HFB
	716	$M \cdot H^+ - RCOOH$	
	520	$M \cdot H^+ - RCOOH - RCO^+ + H^+$	
	215	$RCOOH \cdot H^+$	
	197	$RCOOH \cdot H^+ - H_2O (= RCO^+)$	
4	734	$M \cdot H^+$	M = Naltrexone-3,14-HFB
	548	$M \cdot C_2H_5^+ - RCOOH$	M′ = Naloxone-3-HFB
	524	$M' \cdot H^+$	
	520	$M \cdot H^+ - RCOOH$	
	324	$M \cdot H^+ - RCOOH - RCO^+ + H^+$	
	215	$RCOOH \cdot H^+$	
	169	R^+	
5	328	$M \cdot H^+$	M = Naloxone
6	566	$M \cdot C_2H_5^+$	M = Naltrexone-3-HFB
	538	$M \cdot H^+$	
	342	$M \cdot H^+ - RCO^+ + H^+$	
7	370	$M \cdot C_2H_5^+$	M = Naltrexone
	342	$M \cdot H^+$	

* $R = CF_2CF_2CF_3$.

The partial IR spectra (Fig. 5) contrast the carbonyl absorption regions of naloxone derivatives, IIa and IIIa. It is noted that IIIa exhibits strong ketone absorption at 1736 cm^{-1} whereas IIa lacks this absorption thereby illustrating the role of pyridine in catalyzing enol ester formation.

IIIa and IIIb have longer retention times and give lower ECD responses than the corresponding trisubstituted derivatives (IIa and IIb). Furthermore, it was difficult to prepare IIIa and IIIb without also forming small quantities of IIa and IIb. Thus, IIa and IIb prepared by pyridine catalyzed derivatization with HFBA were considered more suitable for quantitative studies.

The amount of pyridine required to catalyze the formation of IIa and IIb was determined by varying the concentration of pyridine from 0.1 to 5% in benzene. Derivatization was incomplete when 0.1% pyridine in benzene was used. With 5% pyridine in benzene, derivatization was rapid and complete but high ECD backgrounds resulted. Satisfactory chromatograms were obtained with 1% pyridine in benzene.

It was also found that derivatization with 25 μl of HFBA in 50 μl of benzene at 70° for 2 h resulted in the formation of IIa and IIb without the use of pyridine. This procedure, however, resulted in high ECD backgrounds and was therefore not pursued.

Naloxone and naltrexone were also derivatized with TFAA and PFPA both with and without pyridine as a catalyst. The relative retention times and relative response factors of the derivatives are summarized and compared with the HFBA derivatives in Tables I and II. Derivatization with HFBA and PFPA in the presence of pyridine yielded the triesters and in the absence of pyridine primarily the diesters. Reaction with TFAA in the presence or absence of pyridine resulted in the formation of the same derivative for each compound. Furthermore, it is noted that all of the derivatives exhibit comparable electron-capture responses.

Because perfluoroalkyl anhydrides are strong electron absorbers, they must be removed from samples before GLC–ECD or high backgrounds will result. Most investigators remove excess anhydride by evaporation under nitrogen. However, in the procedure described here, evaporation is precluded by the presence of pyridine which reacts with anhydrides to form non-volatile residues which swamp the electron-capture detector. Satisfactory cleanup with minimal decomposition of derivatives was accomplished by washing the reaction mixtures with sodium borate solution.

Since perfluoroalkyl esters are hydrolyzed under mild conditions, the stabilities of derivatives after decomposition of excess anhydride with sodium borate solution were investigated. It was found that naltrexone derivatives decompose more rapidly than the corresponding naloxone derivatives. As a consequence, peak height ratios change with time (Fig. 6). Specifically, IIb decomposed at 23° with a half-life of about 520 min compared to 784 min for IIa. Furthermore, HFB-derivatives are more stable than PFP-derivatives which are in turn much more stable than TFA-derivatives. This observation is consistent with the report of Änggård and Sedvall[16] on the comparative stabilities of several perfluoroalkyl esters of catecholamines. Thus, the rate of change of peak height ratios is slowest for HFB derivatives, faster for PFP derivatives, and fastest for TFA derivatives. Further study of the TFA derivatives was not

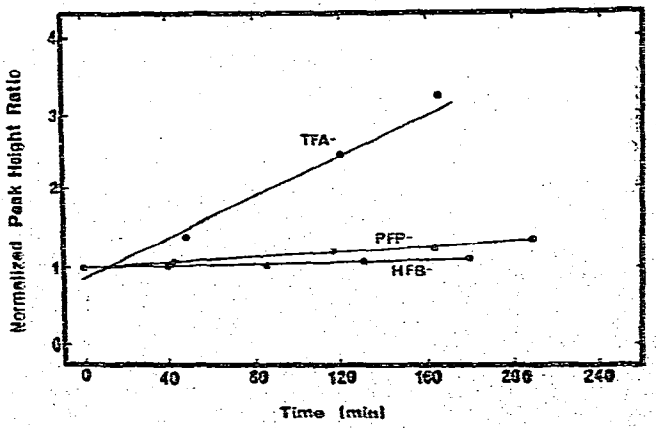


Fig. 6. Plot of normalized peak height ratios against time for naloxone/naltrexone derivatives prepared under pyridine-catalyzed conditions described in the text.

undertaken because of the substantially greater instability of these esters. Because of the time dependence of peak height ratios, excess anhydride was decomposed from each sample just before it was chromatographed. The procedure was carefully timed and samples were not allowed to stand after the anhydride had been decomposed but were chromatographed immediately. These precautions were necessary to obtain linear and reproducible calibration curves for quantitative studies.

It is postulated that the differences in stabilities of naloxone and naltrexone derivatives of the same type (*e.g.* IIa and IIb) are due to differences in basicities of the tertiary amine groups. Initially, it was assumed that the enol esters would be the least stable of the ester groups. However, the distance between the enol ester and the tertiary amine group is so great that differences in the basicities of the amine groups should have no effect on the relative stabilities of the enol esters. Furthermore, the peaks corresponding to the products of enol ester hydrolysis (IIIa and IIIb) do not increase with time as the parent compounds decompose. No hydrolysis products were detected by GLC–ECD. Therefore, it is postulated that the first step in the hydrolytic decomposition of the derivatives involves cleavage of the C-14 ester group as a result of intramolecular base catalysis (Fig. 7). Thus, the rate of hydrolysis increases as the basicity of the tertiary amine increases. Since naltrexone ($pK_a = 8.38$) is more basic than naloxone ($pK_a = 7.94$)[17], naltrexone derivatives are expected to hydrolyze more rapidly. This interpretation is in accord with the observed results. The products of hydrolysis are C-3,6-diesters possessing polar groups at C-14 and having greatly increased retention times.

Because narcotic antagonists such as naloxone and naltrexone are often administered with narcotic agonists, HFB derivatives of morphine, oxymorphone,

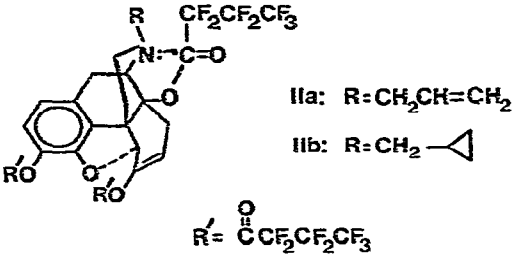


Fig. 7. 3,6,14-HFB esters of naloxone (IIa) and naltrexone (IIb).

TABLE IV

RETENTION TIMES OF DERIVATIVES RELATIVE TO RETENTION TIME OF 9-BROMOPHENANTHRENE

Derivatization catalyzed by pyridine. Separations were carried out on 3% OV-17 on Gas-Chrom Q at 205° with carrier gas flow at 40 ml/min.

Compound	*Relative retention time*		
	TFAA	*PFPA*	*HFBA*
Cyclazocine	—	0.368	0.387
Pentazocine	—	0.429	0.458
Oxymorphone	0.563	0.430	0.441
Morphine	—	1.10	1.13

cyclazocine, and pentazocine were prepared. Relative retention times of derivatives are shown in Table IV. It is noted that all derivatives except the morphine derivative are well resolved from the naloxone and naltrexone derivatives on OV-17.

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