DETERMINATION OF COMPOSITIONS AND CONFIGURATIONS OF *CIS*-A- AND *CIS*-B-OHMEFENTANYL BY HPLC AND ^{1}HNMR

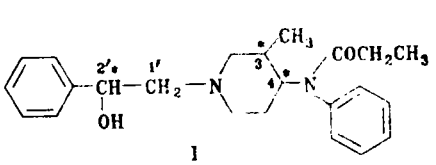
ZX Wang, YC Zhu, N Jiang and RY Ji

(*Shanghai Institute of Materia Medica, Academia Sinica, Shanghai 200031*)

ABSTRACT Ohmefentanyl (1) is an extremely potent analgesic agent with high affinity and selectivity for opioid μ receptors. Using HPLC and ^{1}HNMR spectral analysis, the compositions of two pairs of *cis*-form enantiomers: *cis*-A-1 and *cis*-B-1, were confirmed as a mixture of equal parts of *cis*-(+)-(3*R*,4*S*,2′*S*)-1 + *cis*-(−)-(3*S*,4*R*,2′*R*)-1 and a mixture of equal parts of *cis*-(−)-(3*R*, 4*S*, 2′*R*)-1 + *cis*-(+)-(3*S*, 4*R*, 2′*S*)-1, respectively.

Key words Ohmefentanyl; Stereoisomers; HPLC; ^{1}HNMR

Ohmefentanyl (1, OMF, 7302, *N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide), discovered in our laboratory, is an extremely potent analgesic agent with high affinity and selectivity for opioid μ receptors[1,2]. Due to the presence of three chiral carbons, we expect that there should be eight stereoisomers—four pairs of enantiomers. *Cis*-A-1 and *cis*-B-1 are two pairs of *cis*-form enantiomers of 1. Our previous studies showed that three chiral centers in 1 molecule exhibited important effect on the analgesic potency[3], and the most potent isomer, *cis*-A-1, is approximately 28 and 6300 times more active than fentanyl and morphine respectively in the mice hot plate tests (i. p.). Now, pre-clinical tests of *cis*-A-1 as a narcotic analgesic are in progress. Receptor binding assays demonstrated that *cis*-A-1 had high affinity and selectivity for opioid μ receptors in mouse and rat brain membranes[2,4,5]. Isolated tissue bioassys and autoradiography analysis gave similar results[6～8]. This conclusion was supported by the data of LSP (Ligand Selectivity Profiles) and BSS (Binding Site Signatures) analysis from Goldstein's laboratory. Their results showed that ohmefentanyl was more μ-selective than sulfentanil, about the same as DAGO[9].



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Because opioid receptors distinguish ligands with high stereospecificity, it is necessary to determine compositions and configurations of *cis*-A-1 and *cis*-B-1. However, direct resolution of these two compounds is very difficult. Recently, eight stereoisomers of ohmefentanyl were synthesized and studied[10], and their absolute configurations were determined via X-ray crystallographic analysis[11]. This made the determination of the compositions and configurations of *cis*-A- and *cis*-B-1 possible. ^{1}HNMR analysis of stereoisomers of 1 revealed that there was remarkable difference in their chemical shifts and splitting patterns[12]. Additionally, HPLC method was also used to determine diastereoisomeric purity of *cis*-A- and *cis*-B-1 (Zhu *et al*, unpublished results). Using these methods, determination of the compositions and configurations of *cis*- A- and *cis*- B- ohmefentanyl was accomplished.

EXPERIMENTAL

The melting points were determined in a BÜCHI-510 apparatus and were not corrected. The ^{1}HNMR spectra were recorded with a Bruker AM-400 MHz spectrometer. HPLC analysis was performed on a Shimadzu SPD-10A liquid chromatographic instrument.

Samples

cis-A-OMF, *cis*-B-OMF, four stereoisomers (**1a**～**d**) of *cis*-ohmefentanyl were prepared in our laboratory using reported methods[3,10].

MA A mixture of (+)-*cis*-(3*R*,4*S*,2′*S*)-OMF(**1a**) (8.6 mg) and (−)-*cis*-(3*S*,4*R*,2′*R*)-OMF(**1d**) (8.6 mg) was recrystallized with petroleum ether. White fine needles were obtained, mp 137～139℃.

MB A mixture of (−)-*cis*-(3*R*,4*S*,2′*R*)-OMF(**1b**) (9.6 mg) and (+)-*cis*-(3*S*,4*R*,2′*S*)-OMF(**1c**) (9.5 mg) was recrystallized in petroleum ether. White needles were obtained, mp 114～115℃.

All samples used for HPLC analysis were made up in solution of 50 μg • ml^{-1} in methanol.

HPLC Analysis

The sample (20 μL) was injected into an analytical HPLC column (Lichrosorb Rp 18, 5 μm, 0.5×15 cm), phenacetin was used as reference, eluted at 1.0 ml • min^{-1}, with 60% methanol/40% water/triethylamine (c 0.1 M)/*d*-camphor-10-sulfonic acid (c 0.005 M). The eluate was monitored for optical density at 254 nm.

Retention time: *cis*-A-OMF, 23.083 min; *cis*-B-OMF, 19.745 min; MA, 22.958 min; MB, 19.875 min (Fig 1).

^{1}HNMR analysis

The ^{1}HNMR spectra of *cis*-A-OMF, **1a** and **1b** were recorded in DMSO-d_6 solution, concentration of about 5 mg • 0.5 ml^{-1}, and the solvent peak (δ 2.50) was used as chemical shift reference.

RESULTS AND DISCUSSION

The data from analgesic pharmacological tests, melting points, HPLC and ^{1}HNMR analysis showed that the compositions and configurations of *cis*-A-1 and *cis*-B-1 were (+)-*cis*(3*R*,4*S*,2′*S*)-**1** (**1a**) + (−)-*cis*(3*S*,4*R*,2′*R*)-**1** (**1d**) and (−)-*cis*(3*R*,4*S*,2′*R*)-**1** (**1b**) + (+)-*cis*(3*S*,4*R*,2′*S*)-**1** (**1c**), respectively.

Pharmacological data Analgesic activities of the stereoisomers of ohmefentanyl determined in the mice hot-plate tests showed that the analgesic potency of **1a** was 2 times more potent than that of *cis*-A-1, compound **1b** was 2.7 times more active than *cis*-B-1, while their antipodes, **1d** was inactive. **1c** showed only low analgesic activity[10] (Tab 1). The analgesic effects of *cis*-A-1 and *cis*-B-1 were mediated by **1a** and **1b**, respectively, in agreement with *cis*-A-1 as a racemic mixture of **1a** and **1d**, and *cis*-B-1 as a racemic mixture of **1b** and **1c**.

Melting point Sample MA is a mixture of equal amounts of **1a** and **1d**, and sample MB is a mixture of equal amounts of **1b** and **1c**. Their specific rotation powers were 0°, so MA and MB should be considered as racemic mixtures. The melting point of MA is higher than that of parent compounds **1a** or **1d**, close to the melting point of *cis*-A-1. On the other hand, the melting point of MB is lower than that of **1b** or **1c**, and equal to that of *cis*-B-1 (Tab 1). These facts also showed that *cis*-A-1 had thesame compositions and configuration as MA, and *cis*-B-1 had the same compositions and configuration as MB.

Tab 1 The physico-chemical properties and analgesic activities of *cis*-A-1, *cis*-B-1, MA, MB and 1a～d

Compounds	Configuration	MP (℃)	$[\alpha]_D^{25}$ (MeOH)	HPLC[a] (*Rt* min)	Analgesic Activity[b] ED_{50}(mol·kg^{-1})
1a	(3*R*,4*S*,2′*S*)	117～119	+19.79°		2.89×10^{-9}
1d	(3*S*,4*R*,2′*R*)	117～119	−20.54°		$>2.46\times10^{-5}$
MA(1a+1d)	(3*R*,4*S*,2′*S*)+(3*S*,4*R*,2′*R*)	137～139	0	22.958	
cis-A-1	(3*R*,4*S*,2′*S*)+(3*S*,4*R*,2′*R*)	140～141	0	23.083	6.01×10^{-9}
1b	(3*R*,4*S*,2′*R*)	135～137	−31.91°		1.27×10^{-8}
1c	(3*S*,4*R*,2′*S*)	135～137	+33.15°		2.46×10^{-5}
MA(1b+1c)	(3*R*,4*S*,2′*R*)+(3*S*,4*R*,2′*S*)	114～115	0	19.875	
cis-B-1	(3*R*,4*S*,2′*R*)+(3*S*,4*R*,2′*S*)	116～118	0	19.745	3.45×10^{-8}[c]

a). HPLC condition was described in the Experimental Section. b). See reference 3,10. c). Data of *cis*-B-1 HCl salt, reference 3.

HPLC analysis *cis*-A-1, *cis*-B-1, MA and MB were analyzed by HPLC. Their HPLC spectra were shown in Fig 1, and their retention times were listed in Tab 1. Comparison of these t_R values indicated that t_R value of MA (22.958 min) was equal to t_R (23.083 min) of *cis*-A-1, and t_R (19.875 min) of MB was equal to t_R (19.745 min) of *cis*-B-1. The result was consistent with the

result from melting point analysis.

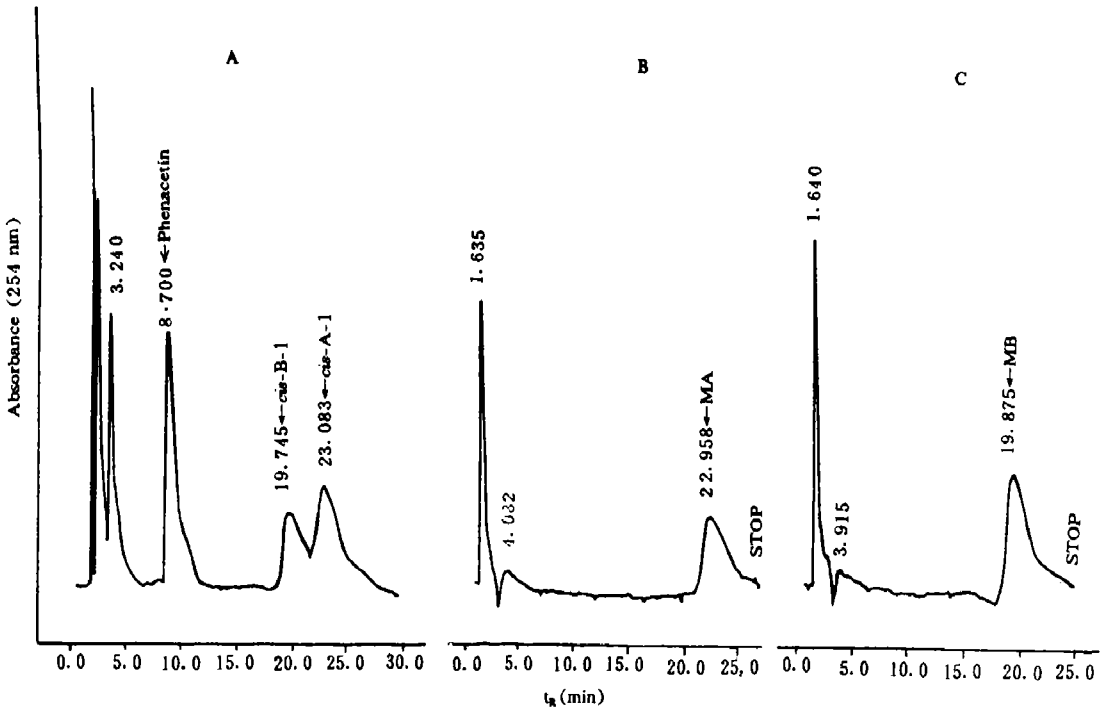

Fig 1 HPLC spectra of *cis*-A-1, *cis*-B-1, MA and MB. (A) *cis*-A-1+*cis*-B-1; the first peak was assigned to *cis*-B-1, and the second peak to *cis*-A-1; (B) MA; (C) MB. HPLC column: Lichrosorb Rp 18, 5 μm, 0.5×15 cm, eluted at 1.0 ml · min^{-1}, with 60% methanol/40% water/triethylamine (c 0.1 M)/*d*-camphor-10-sulfonic acid (c 0.005 M).

^{1}HNMR analysis In general, two enantiomers and their racemic modification have the same ^{1}HNMR spectrum, while there was obvious difference in ^{1}HNMR spectrum between diastereoisomers. The ^{1}HNMR analysis of stereoisomers of **1** revealed their extreme difference in chemical shifts and splitting patterns[12], so comparison of individual ^{1}HNMR spectra should determine the compositions and configurations of *cis*-A-1 and *cis*-B-1. In the present study, the ^{1}HNMR spectra of *cis*-A-1, **1a** and **1b** were studied (^{1}HNMR of *cis*-B-1 was not studied because the sample was scarce). Comparing the ^{1}HNMR spectrum of *cis*-A-1 [Fig 2(a)] with these of (+)-*cis*-(3*R*,4*S*,2′*S*)-1 (**1a**) [Fig 2(b)] and (−)-*cis*-(3*R*,4*S*,2′*R*)-1 (1b) [Figure 2(c)], the spectrum (a) is identical with the spectrum (b) but different from the spectrum (c). The result supported the conclusion that *cis*-A-1 was a racemic modification of **1a** and **1d**, from HPLC analysis and determination of melting point.

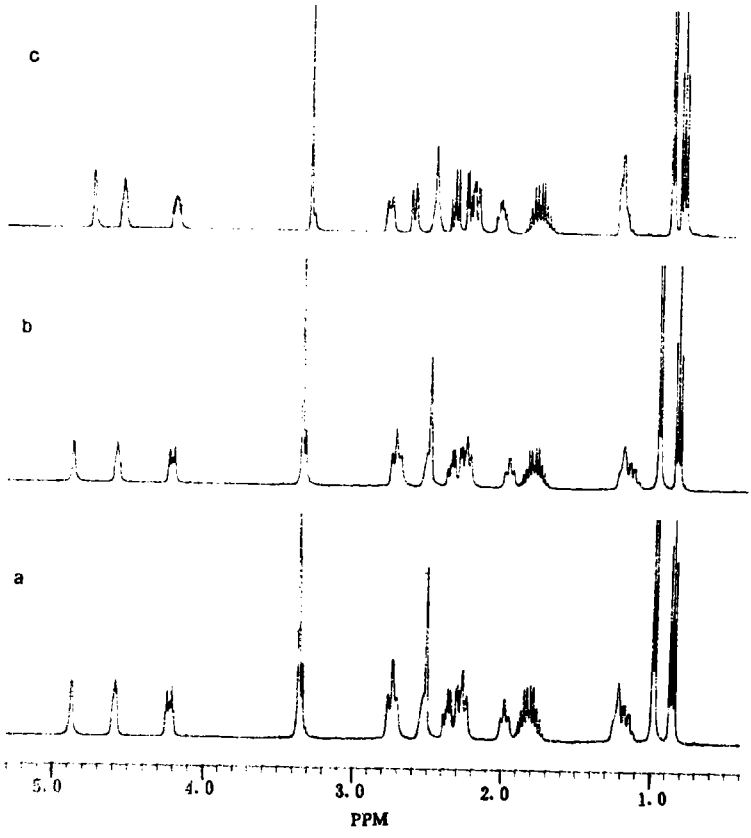

Fig 2 ^{1}HNMR spectra of (a) *cis*-A-OMF, (b) (+)-*cis*-(3*R*,4*S*,2′*S*)-OMF (**1a**) and (c) (−)-*cis*-(3*R*,4*S*,2′*R*)-OMF (**1b**).

To sum up, two pairs of enantiomers of *cis*-ohmefentanyl, *cis*-A-1 and *cis*-B-1, were confirmed as a mixture of equal parts of *cis*-(+)-(3*R*,4*S*,2′*S*)-1 + *cis*-(−)-(3*S*,4*R*,2′*R*)-1 and *cis*-(−)-(3*R*,4*S*,2′*R*)-1 + *cis*-(+)-(3*S*,4*R*,2′*S*)-1, respectively.

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HPLC 和^{1}HNMR 分析确定 *cis*-A-和 *cis*-B-羟甲芬太尼的组成和构型

王智贤 朱友成 蒋 凝 嵇汝运

(中国科学院上海药物研究所,上海 200031)

摘要 羟甲芬太尼(1)是一个强效的镇痛剂和高亲和、高选择性的阿片μ受体激动剂。通过 HPLC 和^{1}HNMR 分析,*cis*-A-1 被确定为由等量的 *cis*-(+)-(3*R*,4*S*,2′*S*)-1 和 *cis*-(−)-(3*S*,4*R*,2′*R*)-1 组成的外消旋体,*cis*-B-1 被确定为由等量的 *cis*-(−)-(3*R*,4*S*,2′*R*)-1 和 *cis*-(+)-(3*S*,4*R*,2′*S*)-1 组成的外消旋体。

关键词 羟甲芬太尼;立体异构体;HPLC;^{1}HNMR