Precursors of the mammalian synthesis of morphine: (+)-salutaridine and (−)-thebaine from (+)-6-demethylsalutaridine, and (−)-*N*-$^{13}CH_3$-thebaine from (−)-northebaine

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Standard samples of pure (+)-salutaridine and (−)-thebaine required to study the mammalian origin of morphine, were prepared from (+)-6-demethylsalutaridine by published procedures and were characterized by CD spectra and physical data. Reductive *N*-methylation of (−)-northebaine afforded (−)-thebaine, and when ^{13}C-labeled formalin was used, (−)-thebaine with a ^{13}C label on the *N*-methyl carbon atom resulted. The latter represents a model procedure to prepare ultimately *N*-$^{14}CH_3$-labeled (−)-thebaine and ^{14}C-labeled congeners.

Morphine *Thebaine* *Salutaridine* *Mammalian biosynthesis*

1. INTRODUCTION

(+)-Salutaridine (**2**) and (−)-thebaine (**5**) are intermediates in the biosynthesis of opioids in the poppy *Papaver somniferum* [1]. After i.v. injection of **2** and **5** in rats, enhanced levels of codeine and morphine were detected, suggesting a similarity in enzymatic degradation of **2** and **5** in both plants and mammals [2]. An unambiguous preparation and purification of both (+)-salutaridine and (−)-thebaine were necessary to rule out contamination and consequently question the endogenous origin of the opioids. Using established procedures, natural (−)-thebaine was converted into **2** through six intermediates, each of which was isolated and purified by recrystallization, and in some cases by chromatography. The nominal absence of codeine and morphine by TLC in the thebaine used as starting material, and the isolation and purification of six intermediates to salutaridine, argue strongly for the absence of codeine and morphine in the salutaridine obtained from this sequence. Salutaridine (**2**) was subsequently reduced to the alcohols **3** and **4** with sodium borohydride. Ring closure of unnatural (−)-salutaridinol II (**4**) afforded pure **5** in high yield. An alternative pathway to **5** employed reductive *N*-methylation of (−)-northebaine (**6**); this method was also utilized with ^{13}C-labeled formalin to give *N*-$^{13}CH_3$-thebaine (**7**). This procedure represents a useful model reaction for the preparation of ^{14}C-labeled thebaine, and ^{14}C-labeled congeners derived from it required to quantitate products obtained in the mammalian biosynthesis of morphine.

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2. MATERIALS AND METHODS

2.1. *Synthesis*

Aluminum oxide used for chromatographic separations was neutral activated Brockmann I grade (Alox) from Aldrich, Milwaukee, WI. Silica gel plates used for TLC were from Analtech, Newark, DE. The solvent system used for TLC analysis was $CHCl_3/CH_3OH/NH_4OH$ (90:10:1) and materials were visualized by exposure to iodine vapors. (−)-Northebaine (**6**) was prepared from natural (−)-thebaine by the method of Schwab [3]. The following procedures used for converting **5** into **2**, and **2** back into **5**, gave the most satisfactory results: diphenol **1** was obtained from **5** [4] by a route developed by Fleischhacker et al. [5], which was found to be simpler and more reliable than the bisulfite-mediated oxidation used by Bjeldanes and Rapoport [6]. Conversion of **1** into **2** was accomplished by protection of the phenolic groups, selective deprotection and *O*-methylation with diazomethane, as described by Horvath and Makleit [7].

(+)-Salutaridine (**2**) was characterized by its CD spectrum shown below. Conversion of **2** into **5** followed methods used by Barton et al. [8,9]. In accord with their findings, reduction of **2** with sodium borohydride in methanol afforded alcohols **3** and **4**, separated by chromatography on Alox. The slower moving unnatural alcohol **4** was cyclized to **5** with DMF-dineopentylacetal, as described by White et al. [10]. CD spectra of natural (−)-salutaridinol I (**3**), (−)-thebaine (**5**) and (−)-codeine are also recorded here. Thebaine prepared from **2** was found to be identical with the natural alkaloid after purification on Alox and crystallization from ethanol. Both samples gave identical physical data, and essentially identical to those reported [4].

Reductive *N*-methylation of **6** with formalin and sodium cyanoborohydride in acetonitrile [11] and purification of **5** as the tartrate salt afforded **5** after usual transformation into the base in very good yield. Repetition of the experiment with ^{13}C-labeled formalin [12] gave (−)-thebaine (**7**), with a ^{13}C label on the *N*-methyl carbon atom. The physical properties of **7** were almost identical to those found for **5**, however, the ^1H-NMR spectrum of **7** showed the expected doublet ($^1J_{^{13}CH}$ = 133 Hz) centered at δ 2.38 for the *N*-$^{13}CH_3$ group. The spectrum also showed 5% *N*-$^{12}CH_3$-thebaine as revealed by a singlet at δ 2.38 due to isotopic impurity in the [^{13}C]formalin employed for reductive alkylation. The chemical ionization MS of **7** showed the expected molecular ion at 313 (M + 1), one mass unit higher than 312 (M + 1) observed for **5**.

2.2. *CD spectral determination*

The CD spectra were recorded in ethanol at room temperature on a Jasco model J-500A recording spectropolarimeter, equipped with a Jasco model DP-500N data processor, and an OKI IF-800 model 30 microcomputer.

The CD spectra are in agreement (within experimental error) with those reported in the literature [13]. The spectral patterns are characteristic for each of these compounds and reveal more fine structure than reported [13]. Our CD spectrum of thebaine exhibits a true Cotton effect at 242/3 nm whereas a shoulder is shown in the literature in the same wavelength area (due to the latest refinement of instrumental techniques such as data averaging, use of microcomputers, etc. one can detect more details).

As mentioned by Crabbe [14], the intensity of the Cotton effects of these compounds is quite high, especially below 250 nm, presumably due to the electric dipole-electric dipole or magnetic dipole-electric dipole coupling between the transition moments of the aromatic chromophore and ethylene, diene or $\Delta^{1,4}$-3-keto chromophores.

3. EXPERIMENTAL

3.1. *6-Demethylsalutaridine (1) from commercial thebaine (5)*

Thebaine (**5**), free of codeine and morphine by TLC, was converted as described [5] in three steps to **1** which showed m.p. 185–187°C ([5]: m.p. 184–186°C).

3.2. *(+)-Salutaridine (2) from 6-demethylsalutaridine (1)*

Preparation of **2** from **1** was accomplished using the four-step sequence of Horvath and Makleit [7]. **2** showed m.p. 201–202°C; $[\alpha]_D^{23}$ +113° (*c* 0.45, MeOH), $[\alpha]_D^{23}$ +87° (*c* 0.49, $CHCl_3$) ([7]: m.p. 197–198°C $[\alpha]_D^{12}$ +114° (*c* 0.5, MeOH)).

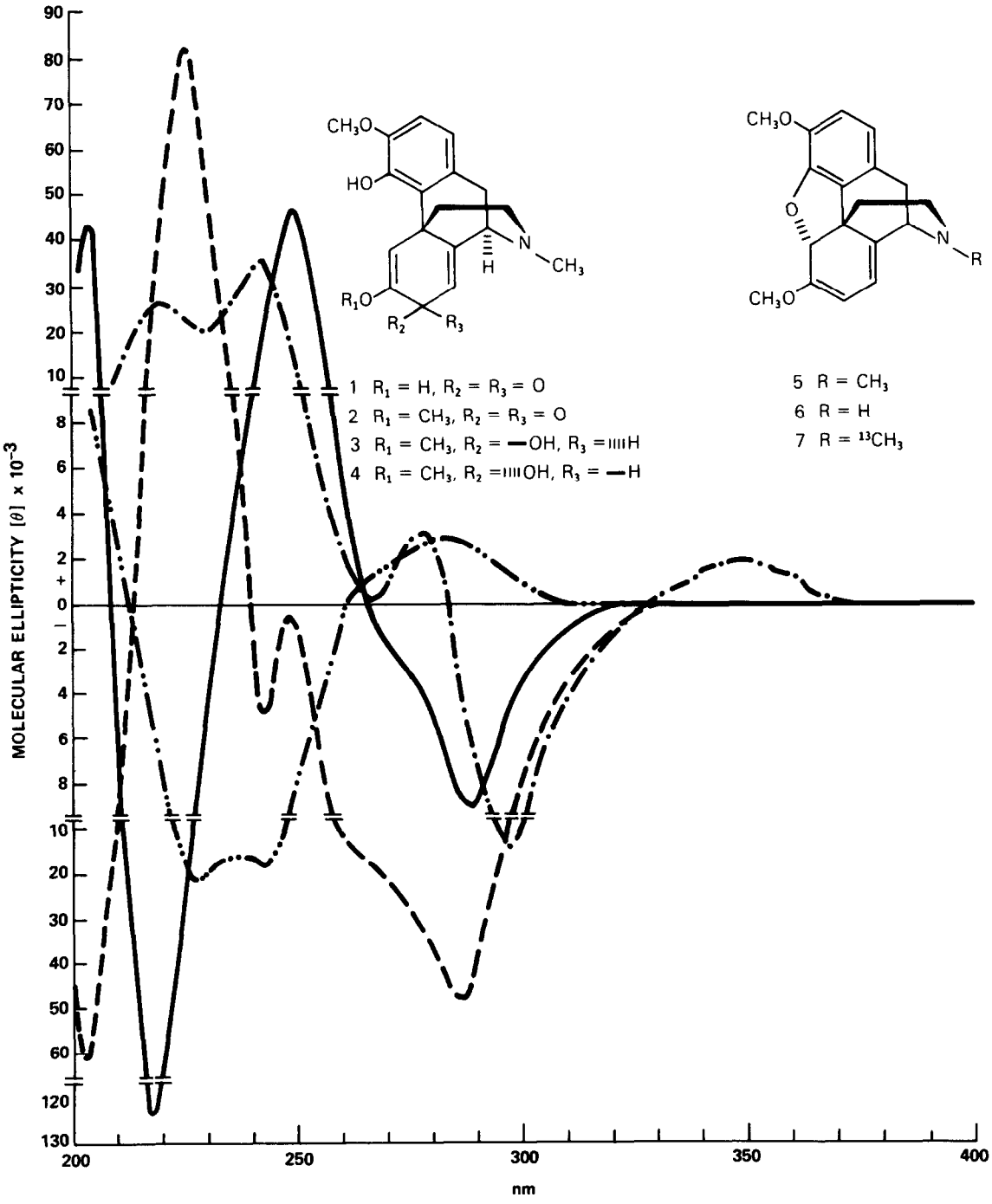

Fig.1. CD spectra in ethanol: (——) codeine, (– – –) thebaine (**5**), (-----) salutaridine (**2**), (------) salutaridinol (**3**).

3.3. *Natural (−)-thebaine (5) from (+)-salutaridine (2)*

Salutaridinol (**4**) II was prepared by $NaBH_4$ reduction and purified by chromatography as in [8,9]. To material (**4**, 250 mg, 0.76 mmol) in chloroform (15 ml) was added DMF-dineopentylacetal [10] (0.65 ml, 2.33 mmol) and the suspension was allowed to stir overnight. Evaporation in vacuo afforded a green residue which was chromatographed on Alox II with chloroform as an eluant, affording 180 mg thebaine (78%). A sample crystallized from ethanol had m.p. 196°C ([4]: 193°C) and $[\alpha]_D^{20}$ −220°C (*c* 2.0, ethanol) ([4]: $[\alpha]_D^{15}$ −219° (*c* 2.0, ethanol)). Analysis: calcd for $C_{19}H_{21}NO_3$ (311.37): C 73.29, H 6.80, N 4.50%; found: C 73.09, H 6.93, N 4.53%.

3.4. *(−)-Thebaine (5) from (−)-northebaine (6)*

A modification of the procedure for *N*-methylation of amines, by Borch and Hassid [11] was used. To a solution of **6** (300 mg, 1 mmol) in CH_3CN (3 ml), 37% formaldehyde solution (0.24 ml, 3 mmol) was added, followed by $NaBH_3CN$ (100 mg, 1.6 mmol) at 0°C. The reaction mixture was allowed to stir at room temperature for 30 min and then neutralized to pH 6–7 by dropwise addition of glacial acetic acid. After stirring for 45 min, the volatiles were removed in vacuo and 2 N KOH (4 ml) was added to the resulting golden syrup. Extraction with $CHCl_3$ (3 × 10 ml) followed by washing of the combined $CHCl_3$ fractions with 0.5 N KOH (1 × 10 ml) and evaporation of solvent in vacuo yielded 310 mg crude **5** as the free base. Thebaine was dissolved in hot MeOH (5 ml) and d-tartaric acid (150 mg, 1 mmol) in MeOH (1 ml) was added, resulting in 400 mg (86%) of white tartrate, m.p. 227–229°C. Conversion to the free base gave crystalline **5**; TLC showed a homogeneous spot identical to an authentic sample of **5**; m.p. 192–193°C ([4]: 193°C); MS (CI) M+1 = 312; $[\alpha]_D^{22}$ −216.5° (*c* 1.09, $CHCl_3$) ([4]: $[\alpha]_D^{23}$ −230° (*c* 5.0, $CHCl_3$)).

3.5. *$^{13}CH_3$-thebaine (7)*

The method described above was followed, substituting 37% formaldehyde with 20% $^{13}CH_3$-formalin [12] (460 mg, 3 mmol). $^{13}CH_3$-thebaine (**7**) was isolated as the d-tartrate in 84% yield of m.p. 227–229°C. Conversion to the free base gave crystalline material; TLC showed the material to be homogeneous and identical with natural thebaine; m.p. 192–193°C; MS (CI) M+1 = 313; $[\alpha]_D^{22}$ −214.8° (*c* 1.07, $CHCl_3$).

4. CONCLUSIONS

Although the amounts of codeine and morphine detected in rat tissues after administration of **2** and **5** are in the femtomolar range, the levels are enhanced as compared to control data and thus their origin is quite possibly of endogenous nature. Quantitation of endogenous opioids resulting from administration of precursors of morphine and codeine would undoubtedly be facilitated with the availability of ^{14}C-labeled compounds. Although *N*-demethylation of opioids is a possible route of metabolism [15], the fact that codeine and morphine with intact *N*-methyl groups could be detected in rat tissue after application of **2** and **5** rationalizes the use of ^{14}C-labeled (−)-thebaine with the label in the *N*-methyl group as a valuable compound. The successful *N*-methylation of (−)-northebaine (**6**) with ^{13}C-labeled formalin, therefore, represents a useful model reaction to prepare such ^{14}C-labeled probes.

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