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SYNTHESIS OF [3-^{14}C]-2′-METHYLRETICULINE AND ITS INCORPORATION INTO ALKALOID FRACTIONS OF *PAPAVER SOMNIFERUM*

THOMAS G. WADDELL and HENRY RAPOPORT*

Department of Chemistry, University of Tennessee at Chattanooga, Chattanooga, TN 37402, U.S.A.; *Department of Chemistry, University of California, Berkeley, CA 94720, U.S.A.

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Key Word Index—*Papaver somniferum*; Papaveraceae; morphine alkaloids; aberrant biosynthesis; 2′-methylreticuline.

Abstract—[3-^{14}C]-2′-Methylreticuline has been synthesized by standard methods. This modified opium alkaloid precursor is efficiently incorporated by aberrant biosynthesis into alkaloid fractions of *Papaver somniferum*, particularly into a highly purified codeine fraction.

INTRODUCTION

Since the discovery that reticuline (**1**) is a precursor of the opium alkaloids in *Papaver somniferum* [1], interest in the synthesis and chemistry of related tetrahydroisoquinolines has remained active [2]. Of special interest are the numerous attempts reported in the literature to convert reticuline into the morphine precursor salutaridine by *in vitro* phenolic oxidative coupling of carbons *p* and *o*′ (in structure **1**). Depending on experimental conditions, however, *o*–*p*′, *p*–*p*′ and *o*–*o*′ coupling predominate [3]. In addition, Rice and co-workers [4, 5] have prepared reticuline congeners in the hope of facilitating and directing oxidative coupling and thus being able to synthesize medicinally valuable alkaloids. With similar motives, it occurred to us that the synthesis of 2′-methylreticuline (**2**) would constitute an attractive means of blocking the *p*′ position and perhaps favor subsequent conversion into a salutaridine derivative. Furthermore, the availability of C-14 labeled **2** would allow examination of a possible biosynthesis of unnatural, methylated opium alkaloids from the corresponding 2′-methyl precursor **2**. The concept of unnatural biosynthetic precursors in *P. somniferum* [6] and *Nicotiana glutinosa* [7, 8] has been previously investigated.

RESULTS AND DISCUSSION

Synthesis of 2′-methylreticuline (2)

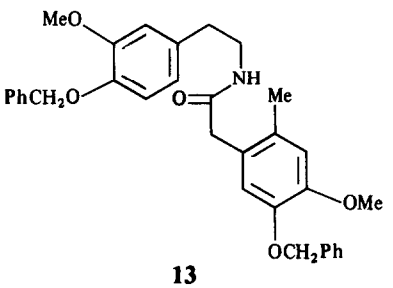
2-Methyl-4-methoxy-5-benzyloxyphenylacetic acid required for a converging synthesis of 2′-methylreticuline was prepared by a modification of a procedure [9] from 3-methoxy-4-hydroxytoluene (see Experimental). In an analogous fashion, vanillin was converted via 3-methoxy-4-benzyloxybenzyl cyanide to the required phenylethylamine.

Coupling of the substituted phenylacetic acid with the phenylethylamine was accomplished (60% yield) in dichloromethane upon treatment with *N*,*N*′-dicyclohexylcarbodiimide. The amide product was subjected to a standard Bischler–Napieralski cyclization ($POCl_3$, toluene) [10] to give the imine hydrochloride **3**, which, as its free base, was converted to the methiodide **4**. Reduction of **4** with sodium borohydride in 2-propanol provided di-*O*-benzyl-2′-methylreticuline (**6**) in 75% yield.

In a parallel fashion, a literature synthesis [1] of di-*O*-benzylreticuline (**5**) was performed in order to compare spectral properties of known compounds with the new intermediates of the 2′-methyl series leading to **6**. Whereas the ^{1}H NMR spectrum of **5** was indicative of a homogeneous compound, that of purified **6** showed two signals each, in a ratio of 2:1, for the aryl methyl group (δ1.85, 2.26) and the *N*-methyl group (δ 2.42, 2.58). Furthermore, the four benzyloxy protons, which appear as a pair of two-proton singlets in **5** (δ4.79, 5.00), are seen as four two-proton singlets (δ4.59, 4.98, 5.47, 5.70) in **6**. These results are consistent with the existence of two non-interconverting conformers of **6** in solution, and in fact, the observation has been made [11] that several 1-benzyltetrahydroisoquinoline compounds exist as two conformers where the C-1 benzyl adopts axial and equatorial orientations. Further evidence for this phenomenon in **6** is provided by its subsequent smooth conversion into the homogeneous 2′-methylreticuline (**2**) in good yield by the action of HCl–ethanol. Since neither **5** nor **2** show any heterogeneity in their ^{1}H NMR spectra, it is apparent that both the 2′-methyl and the benzyloxy groups of **6** contribute to the observed hindered rotation.

The IR and ^{1}H NMR spectra (Experimental) are indicative of the proposed structure **2**. Of particular interest is the three-proton singlet at δ2.02 for the aryl methyl substituent. Compound **2** shows no $[M]^+$ in its mass spectrum, the molecule readily fragmenting between C-1 and C-9 upon electron impact. Exact mass measurements on three intense fragment ions, resulting from this scission, confirm the structure [12]: *m/z* 192 ($C_{11}H_{14}NO_2$, base peak); 151 ($C_9H_{11}O_2$); 177 ($C_{10}H_{11}NO_2$, base peak $-$ Me). Reticuline (**1**) itself gives no $[M]^+$ in the mass spectrum and displays an analogous fragmentation pattern.

The synthesis of the 3-^{14}C labeled **2** was accomplished as described, using $Na^{14}CN$ to produce the benzyl



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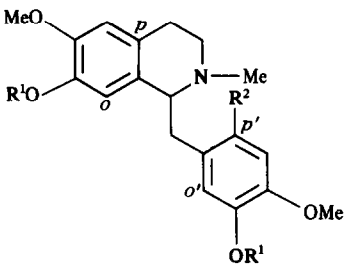

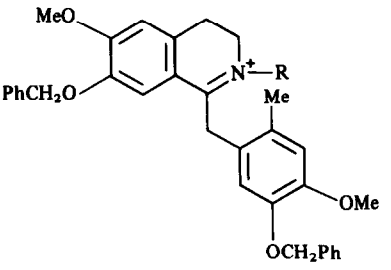

1 $R^1 = R^2 = H$

2 $R^1 = H$, $R^2 = Me$

5 $R^1 = CH_2Ph$; $R^2 = H$

6 $R^1 = CH_2Ph$, $R^2 = Me$

3 R = H

4 R = Me

cyanide (from vanillin): the final product had a specific activity of 5.57×10^7 dpm/mmol. [3-^{14}C]-2′-Methylreticuline was purified by preparative TLC prior to feeding experiments.

Incorporation into P. somniferum–*codeine fraction*

[3-^{14}C]-2′-Methylreticuline was administered to four 63-day-old plants via root feeding from diluted nutrient solution. After 42 hr, the plants were extracted to give a non-phenolic alkaloid fraction (thebaine, codeine; 9.8% incorporation of radioactivity) and a phenolic alkaloid fraction (reticuline, morphine; 0.94% incorporation). Preparative TLC of the non-phenolics followed by preparative GC yielded a highly purified codeine fraction (5.3% incorporation). The mass spectrum of this purified codeine fraction was superimposable on that of authentic codeine. An $[M]^+$ for methylcodeine, which is necessarily present, was not seen since the quantity of this codeine analog represented by the radioactivity in the sample was too small (*ca* 0.2%) to be detected in the mass spectrum. The unlikely possibility that the labeled **2** was extensively degraded and randomly incorporated is precluded by the observed lack of effective incorporation into the phenolic alkaloid fraction. Furthermore, within the non-phenolics, most of the incorporated radioactivity was concentrated in the codeine fraction, a result inconsistent with random incorporation. Since the natural codeine pool is apparently high in *P. somniferum*, more efficient incorporation methods will have to be developed to detect methylcodeine directly by mass spectroscopy.

In summary, [3-^{14}C]-2′-methylreticuline has been synthesized and evidence is presented that *P. somniferum* converts this unnatural precursor into the codeine analog. In addition, the availability of 2′methylreticuline suggests an *in vitro* investigation of its *p* to *o′* oxidative coupling to ring-methylated salutaridine.

EXPERIMENTAL

All mps are uncorr. IR spectra were determined in $CHCl_3$. ^{1}H NMR spectra were determined in $CDCl_3$ with absorptions recorded in ppm downfield from internal TMS. EIMS (probe, 70 eV) were determined on CEC-103 and 110B spectrometers. Radioactivity determinations were performed with a liquid scintillation counter. Silica gel 60 precoated plates (EM Reagents) were used for TLC. Prep. GC was at 220° using argon at 60 ml/min as carrier gas. The column was 1.9 m × 6 mm o.d. with 3% OV-17 on Aeropack-30, 100–120 mesh. The retention time for codeine is 5 min.

4-Methylveratrole was prepared from veratrole by Me_2SO_4–NaOH and purified by distillation, bp 70–74, 0.2 mm (lit. [13] bp 60, 0.2 mm). The product was homogeneous by GC

6-Methylveratraldehyde was obtained by treatment of 4-methylveratrole with DMF and $POCl_3$ [13]. After work-up, the resulting oil crystallized upon trituration with *n*-hexane, mp 71–73° (lit. [13] mp 72–73°). Demethylation (conc. H_2SO_4 at 65–75° for 17 hr) yielded 6-methylisovanillin, mp 150–152° (lit. [14] mp 151–153°), which yielded 2-methyl-4-methoxy-5-benzyloxybenzaldehyde with K_2CO_3 and benzyl chloride. This compound had mp 107–109°. (Found: C, 74.76; H, 6.38 $C_{16}H_{16}O_3$ requires: C, 74.99; H, 6.29%.)

2-Methyl-4-methoxy-5-benzyloxybenzyl alcohol was prepared from the aldehyde ($NaBH_4$ in *i*-PrOH) and had mp 64–67°. IR cm^{-1}: 3670 (OH) ^{1}H NMR: δ7.37 (5H, *m*), 6.95 (1H, *s*), 6.73 (1H, *s*), 5.11 (2H, *s*), 4.51 (2H, *s*), 3.87 (3H, *s*), 2.25 (1H, *s*), 2.30 (3H, *s*). (Found: C, 74.23; H, 6.79. $C_{16}H_{18}O_3$ requires: C, 74.40; H, 7.02%.) This gave the corresponding bromide on treatment

with PBr_3 in Et_2O. It had mp 71–72°. ^{1}H NMR: δ7.28 (5H, *m*), 6.76 (1H, *s*), 6.58 (1H, *s*), 4.95 (1H, *s*), 4.33 (1H, *s*), 3.71 (3H, *s*), 2.24 (3H, *s*). (Found: C, 60.01; H, 5.61; Br, 24.94. $C_{16}H_{17}O_2Br$ requires: C, 59.83; H, 5.33; Br, 24.88%.)

2-Methyl-4-methoxy-5-benzyloxybenzyl cyanide was prepared from the bromide with NaCN in DMF Recrystallization of the product gave mp 81–83°. IR cm^{-1}: 2270 (CN). ^{1}H NMR: δ7.45 (5H, *m*), 6.95 (1H, *s*), 6.78 (1H, *s*), 5.16 (2H, *s*), 3.92 (3H, *s*), 3.58 (2H, *s*), 2.33 (3H, *s*). (Found: C, 76.08; H, 6.27; N, 5.41. $C_{17}H_{17}NO_2$ requires: C, 76.38; H, 6.41; N, 5.24%.) The cyanide yielded 2-methyl-4-methoxy-5-benzyloxyphenylacetic acid by refluxing in KOH in 40% EtOH. This had mp 121–123°. IR cm^{-1}: 1720 (CO). ^{1}H NMR: δ7.36 (5H, *m*), 6.77 (1H, *s*), 6.70 (1H, *s*), 5.07 (2H, *s*), 3.82 (3H, *s*), 3.51 (2H, *s*), 2.25 (3H, *s*). (Found: C, 71.71; H, 6.41. $C_{17}H_{18}O_4$ requires: C, 71.31; H, 6.34%.)

3-Methoxy-4-benzyloxybenzyl bromide. O-Benzylvanillin (prepared as usual, mp 60–63°) was reduced ($NaBH_4$) to the alcohol, mp 71–72° (recrystallized from Me_2CO–hexane). IR cm^{-1}: 3820 (OH). ^{1}H NMR. δ3.82 (3H, *s*), 4.55 (2H, *s*), 5.11 (2H, *s*), 7.35 (5H, *m*), 6.90 (3H, *c*). (Found: C, 73 56; H, 6.55. $C_{15}H_{16}O_3$ requires: C, 73.75; H, 6.60%.) This on treatment with PBr_3 in Et_2O gave the bromide, mp 71–74°.

3-Methoxy-4-benzyloxybenzyl cyanide was prepared from the crude bromide via NaCN in DMF. It had mp 64–66° and showed CN absorption in IR at 2260 cm^{-1}. ^{1}H NMR: δ3.62 (2H, *s*), 3.88 (3H, *s*), 5 13 (2H, *s*), 6.82 (3H, *s*), 7.40 (5H, *s*). (Found: C, 75.57; H, 6.14; N, 5.64 $C_{16}H_{15}NO_2$ requires: C, 75.87; H, 5.97; N, 5.53%.) The cyanide was reduced ($LiAlH_4$ in Et_2O) to β-(3-methoxy-4-benzyloxyphenyl) ethylamine (an oil). The IR spectrum showed two NH absorptions (3440, 3670 cm^{-1}). ^{1}H NMR: δ2.80 (2H, *m*), 3.35 (2H, *br t*), 3.76 (3H, *s*), 4.95 (2H, *s*), 6.62 (*br s*, 3H), 7.21 (5H, *m*).

2-Methyl-4-methoxy-N-(4-benzyloxy-3-methoxyphenethyl)-5-benzyloxyphenylacetamide was prepared from the above amine by treating it with the phenylacetic acid (above) in CH_2Cl_2 in the presence of *N,N′*-dicyclohexylcarbodiimide. The product had mp 122–123°. IR cm^{-1}: 3470 (NH), 1660 (amide I), 1615 (amide II). ^{1}H NMR: δ2.07 (3H, *s*), 3 81 (3H, *s*), 3.84 (3H, *s*), 5.09 (4H, *s*), 6.69 (5H, *m*), 7 35 (10H, *m*), 3.38 (2H, *br s*), 2.40 (2H, *m*), 3.80 (2H, *m*). EIMS: *m/z* 525 [M]$^+$. (Found: C, 75.28; H, 6.68; N, 3.34. $C_{33}H_{35}NO_5$ requires: C, 75.40; H, 6.71, N, 2.67%.)

6-Methoxy-1-(2-methyl-4-methoxy-5-benzyloxybenzyl)-3,4-dihydro-7-benzyloxyisoquinoline methiodide (**4**). The acetamide was cyclized in toluene with $POCl_3$ under reflux. The product formed a hydrochloride. IR cm^{-1}· 3400–2200 (NH), 1650 (C=N). ^{1}H NMR. δ2.30 (3H, *s*), 3.92 (3H, *s*), 4.02 (3H, *s*), 4.88 (2H, *s*), 5.11 (2H, *s*). EIMS: *m/z* 507 [M − HCl]$^+$. (Found: C, 70.87; H, 6.24; N, 3.09. $C_{33}H_{33}NO_4$ HCl H_2O requires: C, 70.52; H, 6.41; N, 2.49%.) This free imine was methylated (MeI) to give **4**, mp 144–148°. The IR showed no NH absorption. ^{1}H NMR: δ2 20 (3H, *s*), 3.48 (3H, *s*), 3.92 (3H, *s*), 4.06 (3H, *s*), 4.98 (4H, *s*) EIMS: *m/z* 521 [M − HI]$^+$, 506 [M − HI − Me]$^+$, 430 [M − HI − C_7H_7]$^+$.

Dl-O-benzyl-2′-methylreticuline (**6**). Compound **4** was reduced with $NaBH_4$ in *i*-PrOH and the oily product (homogeneous on TLC· R_f 0.30, 1:1 HOAc–*i*-PrOH) was purified by chromatography on alumina (activity III) eluting with 30% $CHCl_3$–C_6H_6, mp 89–91°. EIMS. *m/z* 523 [M]$^+$, 282, 241, 191, 162, 91 [cf. [12]]. The ^{1}H NMR spectrum was complex due to the presence of conformers: δ1 85, 2.26 (*s*, ArMe, 8·4 ratio), 2.43, 2.58 (*s*, NMe, 8:4 ratio), 3.76 (*s*, OMe), 4.59, 4.98 (*s*, OCH_2), 5.47, 5.70 (*s*, OCH_2), 7.18 (*s*, aromatic). (Found· C, 76.03, H, 7.54 $C_{34}H_{37}NO_4 \cdot Et_2O$ requires: C, 76.34, H, 7.93%.)

[3-^{14}C]-*2′-Methylreticuline* was prepared by substituting Na^{14}CN for unlabelled NaCN in the preparation of 3-methoxy-4-benzyloxybenzyl cyanide (see above). Subsequent labeled compounds were prepared as described with the following specific activities: acetamide (6.72×10^7 dpm/mmol), **4** (5.57×10^7 dpm/mmol), **2** (5.57×10^7 dpm/mmol). Prior to feedings, **2** was purified by prep. TLC (silica gel: $CHCl_3$–MeOH, 3.1; 2 drops NH_4OH).

Feeding of [3-^{14}C]-*2′-methylreticuline.* Four 63-day-old *P. somniferum* plants were fed [3-^{14}C]-2′-methylreticuline (9.29 $\times 10^5$ dpm) through the roots from nutrient soln [15] contained in a darkened Erlenmeyer flask. The precursor was added in 2 ml 2.5 M HCl followed by enough 0.6 M $NaHCO_3$ for neutralization. After 42 hr, the plants were extracted for alkaloids. Analysis of the nutrient soln indicated that the plants had taken up only 17% ($1\,58 \times 10^5$ dpm) of the label administered.

Isolation of phenolic and non-phenolic alkaloids. The plants were frozen in liquid N_2 and ground to a fine powder in a Waring blender and extracted in $CHCl_3$–*i*-PrOH (3:1) plus 10% Na_2CO_3 (10:3). An additional 10 vol. of $CHCl_3$–*i*-PrOH was added and the supernatant was decanted. The plant mash was re-extracted with 3 × 7.5 vol. solvent plus additional 10% Na_2CO_3 The combined soln was extracted with 0.03 M H_3PO_4 (pH 1). The aq. layer (containing alkaloids) was basified (pH 12) with 8 M KOH and extracted with 5 × 100 ml $CHCl_3$. The $CHCl_3$ extract contained the non-phenolic alkaloids (50 mg; thebaine and codeine). The aq. layer was brought to pH 8.5 with CO_2 and 0.3 M H_3PO_4 and extracted with $CHCl_3$ (5 × 100 ml). The organic layer was dried, filtered, and evapd to give 33 mg of the phenolic alkaloids (reticuline and morphine).

The non-phenolic fraction (15 700 dpm) in MeOH was separated by prep. TLC ($CHCl_3$–MeOH, 3:1, plus 2 drops NH_4OH) and the codeine fraction (R_f 0.30) extracted with MeOH. This material was subjected to analytical and prep. GC and gave a single, sharp, symmetrical peak at the retention time of codeine (5 min). This highly purified fraction (8400 dpm) gave a mass spectrum identical to that of authentic codeine (M$^+$ 299). The phenolic alkaloid fraction showed a lower incorporation of activity (1500 dpm).

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