Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 4, pp. 441-444, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00037-6

A PLE-BASED RESOLUTION OF COCAINE, PSEUDOCOCAINE, AND 6- AND 7-METHOXYLATED COCAINE ANALOGUES

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Abstract: The enzymatic hydrolysis of racemic cocaine and cocaine analogues using pig liver esterase (PLE) is shown to afford a practical means for achieving their chemical resolution. This reaction was found to proceed not only with good enantioselectivity, but with an interesting chemoselectivity as well.

Cocaine abuse is one of the greatest concerns of the public today, and in the United States alone one to three million cocaine abusers are estimated to be in need of treatment, six times the number of heroin addicts.[1-3] With the aim to obtain cocaine antagonists for possible use in abuse treatment, particular attention has been devoted recently to the discovery of cocaine analogues that show high affinity binding to the dopamine transporter, but low potency in the inhibition of dopamine uptake.[4-10] The study of cocaine analogues differing sterically or electronically from the parent structure thus becomes a logical starting point in this quest for a cocaine antagonist.

While considerable SAR information is available concerning modifications to cocaine's nitrogen atom, and to the ester substituents located at the 2- and 3-positions of the tropane ring, less is known about the effects of modification to cocaine's two-carbon bridge. The paucity of pharmacological information on cocaine analogues bearing substitution at positions 6 or 7 is, presumably, a consequence of the greater synthetic difficulty that attends the preparation of these compounds in optically pure form. While cocaine itself exists in eight stereoisomeric forms, the introduction of one additional substituent at the 6- or 7-position produces further synthetic complications, giving rise to sixteen possible stereoisomers.

Although a classical, chemical resolution procedure has been applied to (±)-2-carbomethoxy-3-tropinone,[11-15] most non-racemic analogues of cocaine have been prepared using cocaine itself as the starting material. Because of the limitations of these two approaches, there consequently exists the need for developing other methods for procuring non-racemic cocaine analogues.

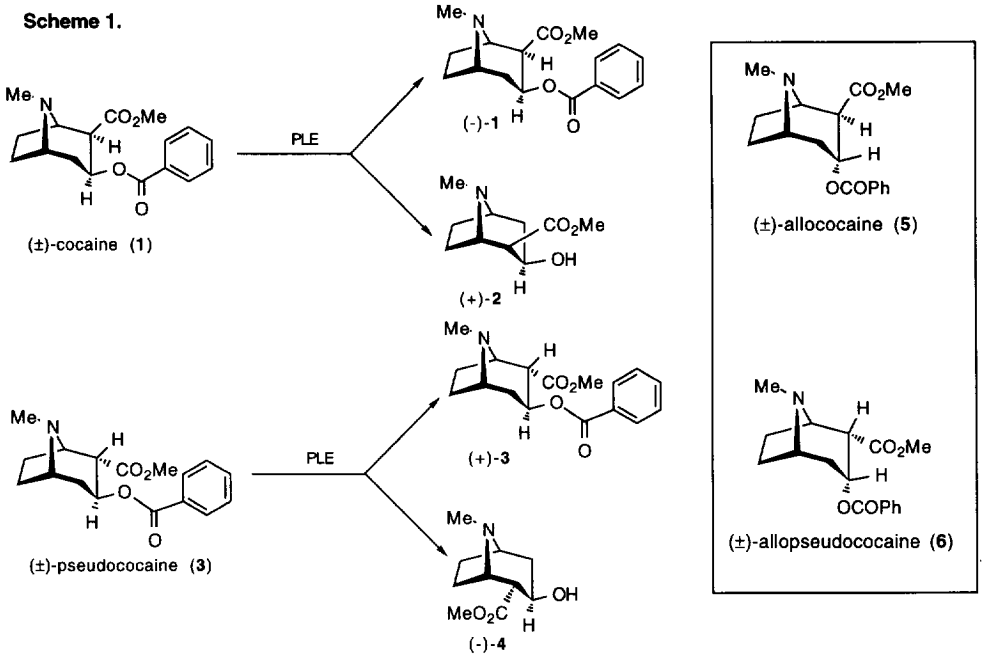
In this Letter we report our preliminary results concerning the PLE-catalyzed resolution of racemic cocaine, pseudococaine (Scheme 1), and its 6- and 7-methoxylated derivatives (Scheme 2). The methoxylated derivatives were synthesized by us previously in racemic form and found to possess interesting pharmacological properties; in particular, some of these methoxylated derivatives were found to antagonize, albeit weakly, cocaine's ability to inhibit dopamine reuptake.[4]

Our efforts to make use of an esterase to bring about the resolution of cocaine and its derivatives were encouraged by observations concerning the metabolism of cocaine both in man and in other species. It has been shown that the major metabolites of cocaine, benzoylecgonine and ecgonine methyl ester, result from the action of serum and liver esterases.[16,17] Additionally, serum pseudocholinesterases hydrolyze cocaine to ecgonine methyl ester. The (1*S*)-isomer of cocaine is hydrolyzed more readily by baboon plasma butyrylcholinesterase than the corresponding (1*R*)-isomer to afford (+)-ecgonine methyl ester.[18] Based upon the foregoing observations and, moreover, our own work in which some of us have shown that PLE has a particular affinity for benzoates,[19] it appeared especially interesting to examine the use of this enzyme in the resolution of (±)-cocaine and its derivatives.

The PLE-catalyzed hydrolysis of cocaine and pseudococaine (Scheme 1) was readily performed in aqueous solution at 37 °C, maintaining the pH at 7. The hydrolysis was continued until one-half equivalent of sodium hydroxide had been consumed. The reaction time was about 1 h for pseudococaine, whereas the hydrolysis of the cocaine molecule required longer reaction times (7 h). In the case of (±)-pseudococaine, preferential hydrolysis of

the (-)-isomer takes place to afford (-)-pseudoecgonine methyl ester [(-)-**4**]. In contrast, for cocaine the (+)-isomer is hydrolyzed at a faster rate. Attempts to effect the PLE-catalyzed hydrolysis of (±)-allococaine (**5**) and allopseudococaine (**6**) failed; the first analogue was recovered unchanged under the reaction conditions described, whereas the second compound was found to undergo slow decomposition.

Scheme 1.



The more rapid rate of hydrolysis of (+)-cocaine by PLE is consistent with an earlier report of the preferential hydrolysis of (1*S*)-cocaine relative to its (1*R*)-counterpart which has been observed in baboon plasma.[18] The preferred hydrolysis of the equatorial -OCOPh group can be rationalized by considering the active-site model first proposed for PLE by the groups of Tamm[20] and Jones.[21-23] In the case of six-membered ring substrates, it has been shown that an equatorial, or pseudo-equatorial ester orientation is preferred by PLE.

In regard to the chemoselectivity of the hydrolysis, i.e., the selective cleavage of the benzoate group rather than the carbomethoxy group, we may advance the hypothesis that the preferred enzyme-substrate complex must allow better geometric alignment of the -OCOPh group with the serine residue present in the catalytic site. In this regard, it may be of interest to note that the use of longer reaction times, and consequently increased amounts of sodium hydroxide, led to the isolation of some ecgonine. However, in consideration of the fact that PLE has generally been used for the resolution of racemates bearing carbomethoxy groups, further experiments will be needed in order to better clarify the mechanism of this selectivity.

Having demonstrated that PLE can bring about the enantioselective hydrolysis of racemic cocaine and pseudococaine, we were interested in applying this reaction to the methoxy-bearing cocaine derivatives **7**, **9**, and **11** (Scheme 2). In short order we found that PLE works efficiently on these derivatives to afford a good separation of the two enantiomers (Table 1). In parallel to the results reported above, the hydrolysis of the pseudococaine-like structures **9** and **11** is faster than that of the cocaine-like structure **7**; the reaction occurs preferably for the (-)-isomers in the case of the pseudococaine derivatives **9** and **11**, but for the (+)-isomer in the case of (±)-6β-methoxycocaine (**7**). The methoxyecgonine methyl ester derivatives (+)-**8**, (-)-**10**, and (-)-**12** obtained after the PLE hydrolysis were simply reacted with benzoyl chloride to provide the final benzoic acid esters in good chemical yield and enantiomeric excess (Table 1).

Scheme 2.

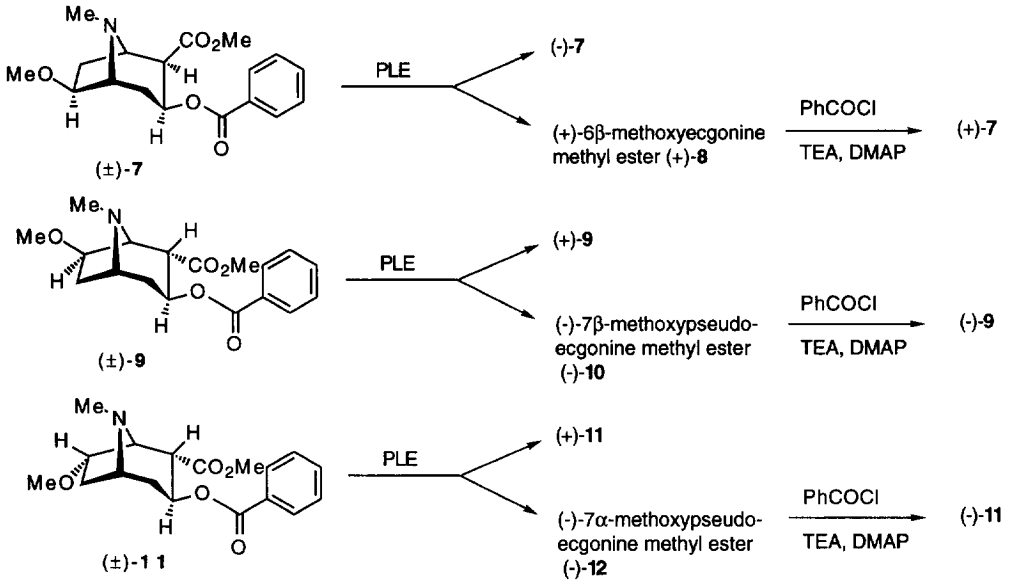


Table 1. Products of the PLE Hydrolysis of Racemic Cocaine and Analogues.[24]

Starting material	Products	Yield (%)	$[\alpha]_D^{20}$	ee (%)
(±)-**1**	(-)-Cocaine (-)-**1**	45	-13.1° (c 2.5, $CHCl_3$) -16° (c 4, $CHCl_3$)[26]	82
(±)-**1**	(+)-Ecgonine Methyl Ester Hydrochloride (+)-**2**	35	+41.5° (c 1.5, MeOH) +52.3° (c 1, MeOH)[13]	71
(±)-**3**	(+)-Pseudococaine Hydrochloride (+)-**3**	91	+42° (c 1.5, H_2O) +41° (c 5, H_2O)[26]	100
(±)-**3**	(-)-Pseudoecgonine Methyl Ester (-)-**4**	85	-22.2° (c 2, H_2O) -22.5° (c 1, H_2O)[13]	95
(±)-**7**	(-)-6β-Methoxycocaine (-)-**7**	35	-16.4° (c 1, MeOH)	82
(±)-**7**	(+)-6β-Methoxyecgonine Methyl Ester (+)-**8**	3	+24.3° (c 2.3, MeOH)	95
(+)-**8**	(+)-6β-Methoxycocaine (+)-**7**	65	+16.1° (c 1, MeOH)	85
(±)-**9**	(+)-7β-Methoxypseudococaine (+)-**9**	60	+35.9° (c 1, MeOH)	99
(±)-**9**	(-)-7β-Methoxypseudoecgonine Methyl Ester (-)-**10**	30	-18.0° (c 3, MeOH)	99
(-)-**10**	(-)-7β-Methoxypseudococaine (-)-**9**	74	-37.0° (c 1, MeOH)	97
(±)-**11**	(+)-7α-Methoxypseudococaine (+)-**11**	55	+46.1° (c 1, MeOH)	97
(±)-**11**	(-)-7α-Methoxypseudoecgonine Methyl Ester (-)-**12**	26	-14.2° (c 2.5, MeOH)	95
(-)-**12**	(-)-7α-Methoxypseudococaine (-)-**11**	67	-46.7° (c 1, MeOH)	97

In summary, application of the PLE-catalyzed hydrolysis reaction to the racemic mixtures of cocaine and its analogues is shown to provide efficient access to the optically pure tropanes. The present methodology thus provides an important means for the procurement of novel, optically pure cocaine derivatives required for pharmacological studies. The good optical yields obtained, and the relatively simple experimental conditions required together with the possibility to carry this reaction out on either the micro or macro scale commends the method to other researchers working in the tropane alkaloid field. The method compares very favorably with alternative procedures, and the extension of these studies to the use of other enzymes for the resolution of tropanes bearing axially oriented benzoate esters is in progress.

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24) Spectral data (IR, ^{1}H NMR, ^{13}C NMR) are the same as reported for the racemic mixtures.[4] The enantiomeric excesses of the products were determined by ^{1}H NMR chiral shift studies employing Eu(Tfc)$_3$ (3-5% molar concentration). As an example, the ^{1}H NMR spectrum of racemic 7β-methoxypseudococaine shows two singlets at δ 4.02 and 3.94 ($COOCH_3$), two singlets at 3.52 and 3.15 (CH_3O), and two singlets at 2.67 and 2.45 (NCH_3). The ^{1}H NMR spectrum of (+)-7β-methoxypseudococaine shows only one singlet at δ 3.90 ($COOCH_3$), one singlet at 3.20 (CH_3O), and one singlet at 2.65 ppm (NCH_3). No traces of the (-)-isomer were detected. On the other hand, the ^{1}H NMR spectrum of (-)-7β-methoxypseudococaine shows one singlet at δ 4.02 ($COOCH_3$), a singlet at 3.48 (CH_3O), and a singlet at 2.44 (NCH_3). The (+)-isomer was not detected. Moreover, the enantiomeric excesses for the ecgonine methyl ester derivatives were also confirmed by their conversion to the corresponding α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters by treatment with (+)-MTPA chloride as described in the literature.[25] The ee for cocaine was calculated by comparison of its optical rotation with that reported in the literature.[26]
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26) Windholz, M. *The Merck Index*, 10th ed.; Merck & Co.; Rahway, N. J., 1983. The rotation of (+)-**3** is 43° in MeOH (c 1) which compares well with the value reported[13] for the (-)-isomer [-43° (c 1, MeOH)].

(*Received in USA* 11 *December* 1995; *accepted* 17 *January* 1996)