Methoxylation of Cocaine Reduces Binding Affinity and Produces Compounds of Differential Binding and Dopamine Uptake Inhibitory Activity: Discovery of a Weak Cocaine "Antagonist"

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Cocaine abuse is one of the greatest concerns of the public today and has therefore become a focus of medical, social, and political leaders.[1,2] Currently a number of treatment strategies are being explored using CNS (central nervous system) agents developed for other afflictions.[3,4] While these agents appear to depress either self-administration or cocaine "craving" under certain circumstances, these studies are still in their early stages, and the efficacy of such treatments has not been established.

The behavioral properties of cocaine, including its abilities to act as a reinforcer, are thought to stem from its ability to inhibit the reuptake of dopamine.[5] Unfortunately, the precise mechanism by which cocaine inhibits dopamine uptake is still uncertain. Several laboratories have shown that cocaine inhibition of dopamine uptake into striatal synaptosomes is consistent with a classic, fully competitive mechanism.[6a-c] However, these data are also consistent with more complex models, including allosteric or partially competitive models and several others involving steric hindrance, distinct but overlapping binding sites, or multiple binding sites in which at least one site is required for both cocaine and dopamine binding.[6d,e]

In recent experiments we have shown that *N*-ethylmaleimide was capable of inhibiting about 95% of the specific binding of [^{3}H]mazindol and that the effect of 10 mM *N*-ethylmaleimide was completely prevented by 10 μM cocaine, while neither 300 μM dopamine nor *d*-amphetamine afforded any significant protection.[7a] Furthermore, a recent study of the structure of the dopamine transporter revealed that aspartate and serine residues lying within the first and seventh hydrophobic putative membrane spanning regions were critical for dopamine uptake, but less so for [^{3}H]CFT binding.[7b] Thus, these data support the hypothesis that a significant portion of the cocaine binding domain on the dopamine transporter is distinct from that of either dopamine or amphetamine. This distinction may be sufficient to allow appropriately designed drugs to prevent cocaine binding without inhibiting dopamine uptake.

On the basis of a large body of SAR work[8] available from our own work and in particular from the work of Carroll and co-workers, we decided to explore the effect of substitution (oxygenation) of cocaine's two-carbon bridge on its binding and functional activity. It appeared that ready access to the racemic 6- and 7-methoxy-substituted analogues of cocaine and pseudococaine could best be achieved by employing chemistry developed nearly 7 decades ago by Willstätter for the construction of cocaine itself (Scheme I).[9]

Thus, the monomethyl ester of acetonedicarboxylic acid, obtained from the reaction of acetonedicarboxylic acid anhydride and methanol at room temperature, was reacted by Mannich type chemistry with methylamine hydrochloride and 2-methoxysuccindialdehyde in a citrate buffer solution to afford the tropinone intermediates **1a-c** in 64% yield. The reaction produced about a 1:1 mixture of (±)-6β-methoxy-2-carbomethoxy-3-tropinone (**1a**) and (±)-7β-methoxy-2-carbomethoxy-3-tropinone (**1b**). Only small amounts (6%) of (±)-7α-methoxy-2-carbomethoxy-3-tropinone (**1c**) were obtained together with **1b**. The 6α isomer could be detected in only trace amounts. When the reduction of the (±)-tropinone derivatives **1a-c** was carried out using sodium amalgam[10] in a sulfuric acid solution at pH 3-4, a mixture of the (±)-methoxyecgonine and (±)-methoxypseudoecgonine derivatives **2-6** was obtained. These five compounds were purified by flash chromatography. Benzoylation of the free bases **2-6** using benzoyl chloride in the presence of TEA and a catalytic amount of DMAP produced in 53-71% yield the desired methoxy substituted cocaine and pseudococaine analogues **7-11** (Scheme II).

Structural assignments for the newly synthesized compounds **7-11** were inferred from ^{1}H and ^{13}C NMR analyses. Positions of all protons in the tropane ring were assigned on the basis of proton-decoupling experiments starting from the diagnostic C_3 proton that appears as a multiplet in the 5.0-5.8 ppm region. In particular, the protons at C_1, C_2, and C_5 have chemical shifts in the range of 3.5-3.9, 2.9-3.1, and 3.2-3.5 ppm, respectively. The C_6 or C_7 proton linked to the methoxy-bearing carbon resonates in the 3.9-4.1 ppm region. The benzoyl protons appear at 7.4-8.0 ppm, while those of *N*-methyl, methoxy, and carbomethoxy groups resonate in the 2.5-2.6, 3.2-3.3, and 3.6-3.7 ppm regions, respectively.

Assignment of the β configuration of the methoxy group for compounds **7-10** was deduced as follows. No coupling is evident between the C_5 proton of compounds **7** and **8** and the neighboring C_6 proton. Likewise, for compounds **9** and **10** no coupling is observed between the C_1 and C_7 protons. In accordance with the Karplus equation, the dihedral angles between the C_5 and C_6 protons and C_1 and C_7 protons must therefore be about 90°, thus defining the β-stereochemistry of the methoxy substituents in these compounds. In contrast, an observed coupling constant of 6.0 Hz between the C_1 and C_7 protons of compound **11** indicates α stereochemistry for the 7-methoxy group. The structure of **7** has been confirmed independently by an X-ray analysis of its hydrochloride salt.[13]

The configuration of the C_2 and C_3 carbon atoms for compounds **7-11** has been unambiguously determined by the size of the coupling constant of the C_2 and C_3 protons (about 6 Hz for the cocaine-like analogues and 10.5 Hz for the pseudococaine-like compounds). The observed values are similar to those reported for cocaine and pseudococaine. Moreover, the ^{13}C NMR spectra for compounds **7-11** are comparable to those reported for cocaine and pseudococaine.[11] The structures of compounds **1a-c** and **2-6** were

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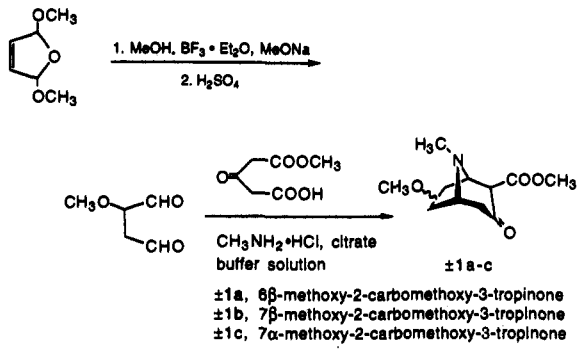
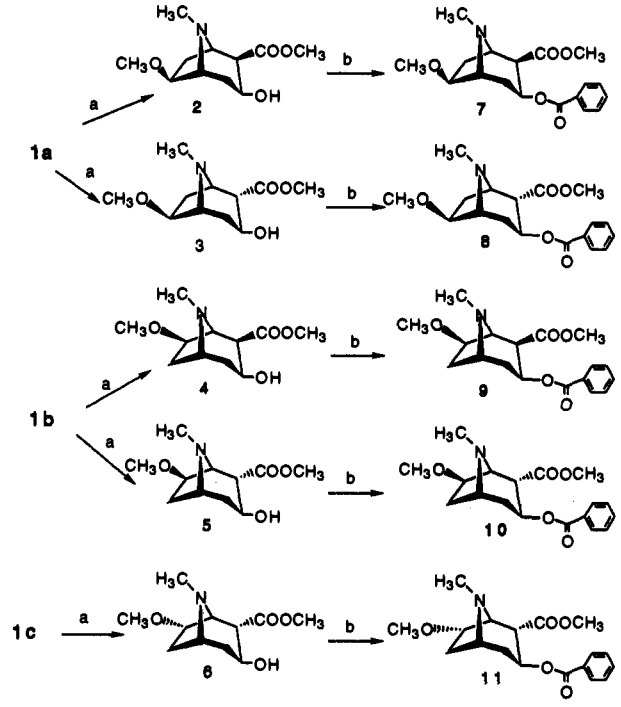
Scheme I



±1a, 6β-methoxy-2-carbomethoxy-3-tropinone
±1b, 7β-methoxy-2-carbomethoxy-3-tropinone
±1c, 7α-methoxy-2-carbomethoxy-3-tropinone

Scheme II. Synthesis of Some Oxygenated Analogues of Racemic Cocaine[a]



[a]Reagents: (a) NaHg, H_2SO_4; (b) benzoyl chloride, Et_3N, DMAP.

Table I. Binding and Dopamine Uptake Data for Compounds 7–11

compound	K_i (μM) [^{3}H]dopamine uptake	K_i (μM) [^{3}H]mazindol binding
cocaine	0.32 ± 0.01	0.28 ± 0.06
pseudococaine	13.8 ± 1.5	10.4 ± 0.3
(±)-7	68 ± 5	98 ± 12
(±)-8	510 ± 110	190 ± 11
(±)-9	6.1 ± 0.2	4.2 ± 0.1
(±)-10	110 ± 4	45 ± 5
(±)-11	200 ± 70	54 ± 3

not assigned directly from NMR experiments, but rather their structures followed from the structural assignments made for compounds 7–11.

The binding and dopamine uptake data for this series of five compounds is presented in Table I. It is apparent from these data that the addition of a methoxy group to cocaine (compounds 7 and 9) leads to a 10–100-fold reduction in activity. This was somewhat surprising to us, since we had imagined that the cocaine recognition site might engage the right-hand portion (the three-carbon bridge comprised of C_2–C_4) of cocaine, while leaving the backside (the two-carbon bridge) free. This obviously is not the case, and we must view the receptor topography as also encompassing the region circumscribed by this two-carbon bridge.

Table II. Effects of Compounds 8 and 11 on Cocaine Inhibition of [^{3}H]DA Uptake

compound	concn, μM	N	cocaine IC_{50} (nM)[a] experimental	theoretical,[a] IC_{50}'
cocaine		12	336 ± 12	
(±)-8	500	5	1022 ± 63	933 ± 16
(±)-11	50	3	501 ± 13	460 ± 22
(±)-11	200	3	936 ± 19*	713 ± 34

[a] For two inhibitors acting at the same site. $IC_{50}' = K_c[1 + [DA]/K_{DA} + [8]/K_8]$ where K_c and K_8 are the equilibrium dissociation constants for cocaine and the putative antagonists (8 or 11), respectively, calculated from the Cheng–Prussof correction for competitive inhibitors, using the IC_{50} values found for cocaine in each individual experiment and the mean IC_{50} of 3–5 experiments for 8 (or 11). K_{DA} is the Michaelis constant for dopamine uptake in our hands (50 nM, Gifford, Bergmann, and Johnson, *Drug Alcohol Depend.* **1993**, *32*, 65–71. [DA] and [8] are the molar concentrations of dopamine and 8 (or 11) used in each experiment. *Significantly different from the theoretical value for two inhibitors acting at the same site, $p < 0.01$ (Student's t-test).

Perhaps the most curious feature of this series of racemic compounds relates to the discrepancy in binding and dopamine uptake inhibition for compounds 8 and 11. The K_i for the binding of compound 11 is about 4 times smaller than its K_i for inhibition of dopamine reuptake, and 2.5-fold in the case of 8. The data provided for pseudococaine in Table I show that this difference does not relate solely to the α stereochemistry of the carbomethoxy group. Compounds 8 and 11 may therefore be capable of countering to some extent the effects of cocaine. Results from experiments addressing this possibility using these compounds are presented in Table II.

Although compound 8, at 500 μM, shifted the cocaine inhibition curve about 3-fold to the right, as evidenced by the increase in cocaine's IC_{50} value from 336 to 1022 μM, this shift is not significantly different from that expected on theoretical grounds for two inhibitors acting at the same site.[12a] On the other hand, compound 11 produced a concentration-dependent rightward shift in the cocaine inhibition curve that was statistically greater than the theoretical value. Although the difference is only about 30%, it does support the idea that it may be possible to design a functional antagonist of cocaine by modifying cocaine's structure.

The mechanism of this apparent antagonism is unknown and additional work is needed to confirm and extend these findings. However, at this time it is possible to suggest that the inhibition of [^{3}H]dopamine uptake by cocaine in the presence of 11 does not result from an action of cocaine and 11 at two completely independent sites. If this were the case, the presence of 11 would be expected to shift the normalized cocaine inhibition curve to the right only slightly under the conditions of this experiment,[12b] rather than the almost 3-fold shift observed here. In addition, the apparent weak antagonism cannot reasonably be thought of as the effect of a "partial agonist", as compound 11 was able to completely inhibit [^{3}H]dopamine uptake with a Hill slope not significantly different from 1. Thus, it appears that 11 may bind to a site (or sites) that results in inhibition of [^{3}H]dopamine uptake and at the same time allosterically diminishes cocaine binding. The interpretation of these data is not straightforward, but they suggest that a more detailed pharmacological analysis of this compound and other C_6- and C_7-modified cocaine and WIN analogues could lead to a more robust antagonism

of the effects of cocaine via a novel mechanism. It is also obvious that the synthesis and study of optically pure 11 is warranted, and this work is currently in progress.

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Supplementary Material Available: Spectral and analytical data for 7–11 and complete X-ray data report for compound 7 (18 pages). Ordering information is given on any current masthead page.

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(13) The author has deposited the atomic coordinates for compound 7 with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.