

## Communications to the Editor

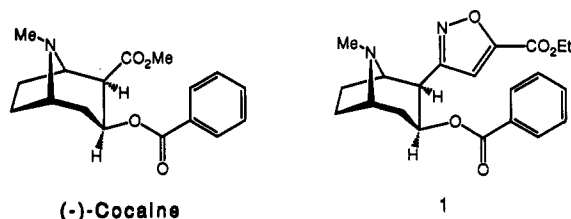
### Structure-Activity Relationship Studies of Cocaine: Replacement of the C-2 Ester Group by Vinyl Argues against H-Bonding and Provides an Esterase-Resistant, High-Affinity Cocaine Analogue

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We have recently described efforts directed toward delineating structure-activity relationships for cocaine in an effort to identify a possible antagonist of cocaine's action at the dopamine re-uptake transporter.<sup>1</sup> During these efforts we discovered that this cocaine-recognition site is rather promiscuous in its ability to accept groups substantially different from cocaine's C-2 carbomethoxy substituent. The carbethoxyisoxazole 1, for example, was found to be about 2-fold more potent than cocaine in both [<sup>3</sup>H]mazindol binding and [<sup>3</sup>H]dopamine uptake studies.



This finding and others made us question the idea that specific hydrogen bond donor groups are present within the cocaine recognition site for binding to the carbomethoxy group (Figure 1).<sup>2</sup> To explore this point further, we chose to investigate the activity of the four new cocaine analogues, 2a-d, in which the C-2 ester group was replaced by a vinyl group incapable of any strong hydrogen bonding.

**Chemistry.** The vinyl compounds 2a and 2b were synthesized readily from the unstable aldehyde 4, a compound obtained by Swern oxidation of the alcohol 3, which is available in optically pure form from (-)-cocaine as shown in Scheme I.<sup>1</sup> After the Wittig reaction of 4, the silyl group is cleaved by the action of fluoride ion, and the free hydroxyl group then is benzoylated.

To obtain compounds 2c and 2d, (*p*-chlorophenyl)-magnesium bromide was added to anhydroecgonine methyl

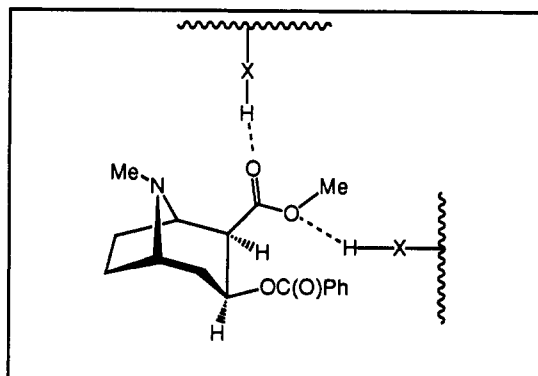
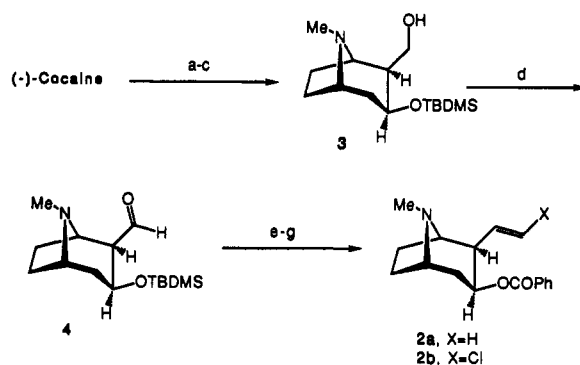


Figure 1. Illustration of possible hydrogen bonding to the C-2 ester group.

Scheme I<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) conc. H<sub>2</sub>SO<sub>4</sub>, MeOH, reflux, 18 h; (b) TBDMSCl, imd., rt, 20 h; (c) Dibal-H, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C, 4 h; (d) Swern oxidation; (e) XCH=PPh<sub>3</sub>; (f) F<sup>-</sup>; (g) PhCOCl, pyr.

ester (5) as described by Carroll et al. in their preparation of additional analogues of the "Win" series of compounds.<sup>3</sup> The 2 $\beta$ -substituted isomer was separated from the 2 $\alpha$ -isomer, and the ester converted to aldehyde in two steps by reduction to alcohol followed by Swern oxidation. Lastly, reaction of this aldehyde with methylenetriphenylphosphorane gave the vinyl analogue 2c, whereas reaction with (chloromethylene)triphenylphosphorane afforded 2d (Scheme II).<sup>4</sup>

**Pharmacological Results.** The four new analogues were tested for their ability to displace [<sup>3</sup>H]mazindol binding<sup>5</sup> from rat striatal membranes as well as to inhibit high-affinity uptake of [<sup>3</sup>H]dopamine into striatal nerve

(3) (a) Carroll, F. I.; Gao, Y.; Rahman, M. A.; Abraham, P.; Parham, K.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis, Ligand Binding, QSAR, and CoMFA Study of 3 $\beta$ -(*p*-Substituted phenyl)tropane-2 $\beta$ -carboxylic Acid Methyl Esters. *J. Med. Chem.* 1991, 34, 2719-2725. (b) Clarke, R. L.; Daum, S. J.; Gambino, A. J.; Aceto, M. D.; Pearl, J.; Levitt, M.; Cumiskey, W. R.; Bogado, E. F. Compounds Affecting the Central Nervous System. 4. 3 $\beta$ -Phenyltropane-2-carboxylic Esters and Analogs. *J. Med. Chem.* 1973, 16, 1260-1267.

(4) To ensure the  $\beta$ -stereochemistry of the 2-vinyl substituent of our analogues, the corresponding 2 $\alpha$ -chlorovinyl isomer of 2d was synthesized independently from the known 2 $\alpha$ -ester. The <sup>1</sup>H NMR spectrum of the 2 $\alpha$ -chlorovinyl isomer showed striking chemical shift differences from that of the 2 $\beta$ -isomer (e.g., =C(H)Cl appears at  $\delta$  = 5.47 in the  $\beta$ -isomer and at 5.83 in the  $\alpha$ -isomer).

(5) (a) Javitch, J. A.; Blaustein, R. O.; Snyder, S. H. [<sup>3</sup>H] Mazindol Binding Associated with Neuronal Dopamine and Norepinephrine Uptake Studies. *Mol. Pharmacol.* 1984, 26, 35-44. (b) Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine Receptors on Dopamine Transporters Are Related to Self-Administration of Cocaine. *Science* 1987,

<sup>†</sup> Mayo Foundation for Education and Research.

<sup>†</sup> University of Texas.

(1) Kozikowski, A. P.; Xiang, L.; Tanaka, J.; Bergmann, J. S.; Johnson, K. M. Use of Nitrile Oxide Cycloaddition (NOC) Chemistry in the Synthesis of Cocaine Analogues; Mazindol Binding and Dopamine Uptake Studies. *Med. Chem. Res.* 1991, 1, 312-321.

(2) Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Cocaine Receptor: Biochemical Characterization and Structure-Activity Relationships of Cocaine Analogues at the Dopamine Transporter. *J. Med. Chem.* 1992, 35, 969-981.

## Scheme II

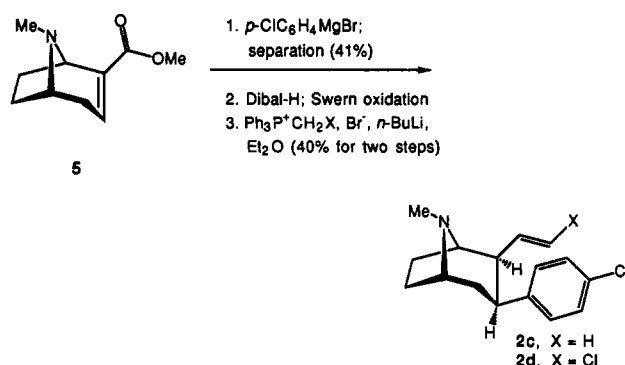


Table I. Dopamine Uptake and Mazindol Binding of Cocaine Analogues 2a-d and 6

cocaine analogue	$\text{IC}_{50}$ (nM), mean $\pm$ SE (N)	
	$[^3\text{H}]$ mazindol binding	$[^3\text{H}]$ dopamine uptake
(-)-cocaine	$580 \pm 70$ (3)	$570 \pm 180$ (2)
2a	$1730 \pm 550$ (3)	$1120 \pm 390$ (4)
2b	$222 \pm 49$ (7)	$368 \pm 190$ (4)
2c	$1.2 \pm 0.3$ (5)	$2.8 \pm 0.6$ (5)
2d	$0.7 \pm 0.1$ (3)	$1.3 \pm 0.3$ (3)
6	$1.6 \pm 0.1$ (3)	$3.2 \pm 0.1$ (3)

endings (synaptosomes) in accordance with protocols described previously.<sup>1,6</sup> Mazindol was selected as the ligand to label the cocaine recognition site because of its known potency correlations with  $[^3\text{H}]$ dopamine uptake and reinforcing properties and similarities to cocaine binding.<sup>5</sup> These data are presented in Table I. Additionally, for comparison purposes, data are presented for (-)-cocaine and for 3 $\beta$ -(*p*-chlorophenyl)tropane-2 $\beta$ -carboxylic acid methyl ester (6).

**Drug Discrimination Studies.** The results of substitution and temporal tests conducted in rats trained to discriminate cocaine (10 mg/kg) from saline using a standard two-lever, water-reinforced FR 20 drug discrimination task are presented in Tables II and III.<sup>10</sup> The percentage of drug-appropriate responding after various doses of cocaine (0.625–10 mg/kg) increased in a dose-dependent manner (Table II). The dose of cocaine predicted to engender 50% cocaine-lever responding ( $\text{ED}_{50}$ ) was 1.7 mg/kg. Response rates were fairly stable across all test doses of cocaine. Testing at several intervals

Table II. Results of Cocaine Dose-Response and Temporal Tests in Animals Trained To Discriminate Cocaine (10 mg/kg) from Saline

drug	dose (mg/kg)	postinjection interval <sup>a</sup> (min)	% drug responses <sup>b</sup>	rate <sup>c</sup> (responses/min)	n/N <sup>d</sup>
Dose-Response Tests					
saline		15	$9 \pm 1$	$28 \pm 4$	7/7
cocaine	0.62	15	$6 \pm 2$	$27 \pm 5$	7/7
	1.25		$44 \pm 18$	$21 \pm 5$	7/7
	2.5		$61 \pm 16$	$32 \pm 16$	7/7
	5.0		$98 \pm 2$	$89 \pm 16$	7/7
	10.0		$99 \pm 1$	$76 \pm 13$	7/7
$\text{ED}_{50} = 1.7 \text{ mg/kg}$					
Temporal Tests					
cocaine	10.0	5	$85 \pm 13$	$28 \pm 8$	7/7
		15	$99 \pm 1$	$76 \pm 13$	7/7
		30	100	$61 \pm 11$	7/7
		60	$81 \pm 14$	$23 \pm 4$	7/7
		120	$31 \pm 18$	$28 \pm 7$	7/7

<sup>a</sup> Time between drug injection and placement in operant chamber for the start of the substitution test. <sup>b</sup> Mean percentage of cocaine-appropriate responses ( $\pm$ SEM) during the test session. <sup>c</sup> Mean number of responses per min ( $\pm$ SEM) prior to completion of the first FR 20 observed during the test session. <sup>d</sup> n/N: number of animals (n) completing 20 responses on either lever out of the number of animals tested (N).

Table III. Results of Substitution Tests with 2c following Different Injection Intervals in Animals Trained To Discriminate Cocaine (10 mg/kg) from Saline<sup>a</sup>

drug	dose (mg/kg)	postinjection interval (min)	% drug responses	rate (responses/min)	n/N
2c	0.05	30	$19 \pm 4$	$29.6 \pm 9$	7/7
	0.1		$40 \pm 17$	$37.1 \pm 6$	7/7
	0.2		$83 \pm 10$	$26.2 \pm 6$	7/7
	0.4		$99 \pm 1$	$52.8 \pm 9$	7/7
	0.8		$93 \pm 5$	$33.5 \pm 9$	7/7
$\text{ED}_{50} = 0.11 \text{ mg/kg}$					
2c	0.05	120	$22 \pm 14$	$18.4 \pm 6$	7/7
	0.1		$75 \pm 16$	$25.7 \pm 5$	7/7
	0.2		100	$46.8 \pm 9$	7/7
	0.4		$72 \pm 28$	$24.9 \pm 18$	3/5 <sup>b</sup>
	0.8		95	7.7	1/7 <sup>b</sup>
$\text{ED}_{50} = 0.08 \text{ mg/kg}$					
2c	0.05	240	$14 \pm 14$	$28.3 \pm 5$	7/7
	0.1		$61 \pm 17$	$23.5 \pm 4$	7/7
	0.2		$98 \pm 1$	$47.9 \pm 10$	7/7
	0.4		$91 \pm 9$	$23.6 \pm 18$	4/5 <sup>b</sup>
	0.8		100	$39.1 \pm 1$	3/7 <sup>b</sup>
$\text{ED}_{50} = 0.09 \text{ mg/kg}$					

<sup>a</sup> See Table II for explanation of table headings. <sup>b</sup> Administration of 2c induced an intense stereotypic response and some animals failed to complete the test session.

(5–120 min) following the administration of cocaine (10 mg/kg) indicated that cocaine injection elicited a complete cocaine-like response within 5 min that lasted for approximately 60 min. When tested 120 min following injection, cocaine elicited primarily saline-appropriate responding (Table II).

Administration of 2c (0.05–0.8 mg/kg) engendered a dose-dependent substitution for the cocaine cue (Table III). The  $\text{ED}_{50}$  predicted for 2c was 0.11 mg/kg following the 30-min injection period. Variation of the postinjection interval (30–240 min) indicated that 2c has a long duration of action (Table III). Administration of 0.2 and 0.4 mg/kg of 2c induced complete cocaine-like responding in all animals 30 min after injection; however, these doses induced intense stereotypy 120 and 240 min after administration which resulted in behavioral disruption (i.e., the animals were unable to complete the FR 20 test session).

237, 1219–1223. (c) Reith, M. E. A.; Selmeci, G. Radiolabeling of Dopamine Uptake Sites in Mouse Striatum: Comparison of Binding Sites for Cocaine, Mazindol, and GBR 12935. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1992, 345, 309–318.

(6) Yi, S.-J.; Johnson, K. M. Effects of Acute and Chronic Administration of Cocaine on Striatal  $^3\text{H}$ -Dopamine Uptake, Compartmentalization and Release. *Neuropharmacology* 1990, 29, 475–486.

(7) (a) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, 1991. (b) Bartlett, P. A.; Marlow, C. K. Evaluation of Intrinsic Binding Energy from a Hydrogen Bonding Group in an Enzyme Inhibitor. *Science* 1987, 235, 569–576.

(8) Andrews, P. R.; Craik, D. J.; Martin, J. L. Functional Group Contributions to Drug-Receptor Interactions. *J. Med. Chem.* 1984, 27, 1648–1657. For comparison purposes, we note that methylation of the hydroxyl group of the neuroleptic 6-methoxysalicylamide results in reduction of binding by 130-fold due to loss of hydrogen bonding: De Paulis, T.; Kumar, Y.; Johansson, L.; Ramsby, S.; Florvall, L.; Hall, H.; Angeby-Möller, K.; Ögren, S.-O. Potential neuroleptic agents. 3. Chemistry and antidopaminergic properties of substituted 6-methoxysalicylamides. *J. Med. Chem.* 1985, 28, 1263–1269.

(9) For a discussion of cocaine biochemical probes, see pages 975–976 of ref 2.

(10) Callahan, P. M.; Appel, J. B.; Cunningham, K. A. Dopamine  $\text{D}_1$  and  $\text{D}_2$  mediation of the discriminative stimulus properties of *d*-amphetamine and cocaine. *Psychopharmacology* 1991, 103, 50–55.

Response rates were stable across the different test doses of **2c**, with the exception of the highest dose (0.8 mg/kg) of **2c** which reduced response rates, possibly due to the observed stereotypy.

**Discussion.** As is apparent from the data of Table I, the vinyl analogue **2a** of cocaine is not substantially less active than the parent structure; in the dopamine-uptake experiment it is only 2-fold less potent. By appending a chlorine atom to the  $\beta$ -position of the vinyl group, one obtains an analogue **2b** which in mazindol binding is about 2-fold more potent than cocaine.

Compound **2c** was selected for synthesis in light of the fact that  $3\beta$ -phenyltropane- $2\beta$ -carboxylic acid methyl ester and its para-substituted phenyl derivatives are the most potent cocaine analogues reported to date.<sup>3</sup> The *p*-chlorophenyl compound **6** synthesized previously by Carroll and co-workers, for example, was reported to exhibit an  $IC_{50}$  of 1.17 nM in displacing  $3\beta$ -(*p*-fluorophenyl)tropane- $2\beta$ -carboxylic acid methyl ester binding from rat striatal membranes.<sup>3</sup>

Compound **6** exhibited  $IC_{50}$ s of 1.6 nM in our mazindol binding study and 3.2 nM in the dopamine-uptake study. Compound **2c** is thus slightly more potent than its ester counterpart **6**.

Overall, the present results fail to support the existence of a hydrogen-bonding pattern of the type illustrated in Figure 1. If we consider a single hydrogen bond to contribute about 1.5–3 kcal mol<sup>-1</sup> to the free energy of interaction of cocaine with its recognition site,<sup>7</sup> then loss of a single hydrogen bonding interaction should lead to at least a 10–100-fold increase in the measured  $IC_{50}$  value for mazindol binding (this estimate fails to take into account the additional loss in affinity of the vinyl compound due to the absence of the methoxy group, a second possible H-bond acceptor group).<sup>8</sup> The 3-fold increase in the  $IC_{50}$  of (–)-cocaine is thus less than anticipated. The approximate 2-fold higher affinity of **2b** compared to cocaine is more likely due to the hydrophobic or dipole interaction contributed by the single chlorine atom rather than to hydrogen bonding, since chlorine is a relatively poor hydrogen-bond acceptor group.

Perhaps most informative is the comparison between compounds **2c** and **6**. Compound **2c** is slightly more potent than the "Win" type compound **6** in spite of the fact that

an unfunctionalized vinyl group serves as the ester group surrogate. Hydrogen bonding is, of course, the strongest when it involves an interaction between a proton and an electronegative atom (N, O, F). Although a weak interaction between the double bond of the vinyl group of **2c** and the H-bond donor group could be postulated, such an interaction cannot explain the similar  $IC_{50}$  values of **2c** and **6**. The presence of the additional chlorine atom on the vinyl group of **2d** further increases binding affinity, doubling the potency of **2d** relative to **2c**. Accordingly, it is unlikely that the hydrogen-bonding interactions depicted in Figure 1 are operative in the binding of cocaine to its recognition site on the dopamine transporter, unless compounds **2a–d** should have a different binding modality available to them.

The data obtained from the drug discrimination studies and presented in Tables II and III further confirm that **2c** is at least 20-fold more potent than cocaine in vivo following intraperitoneal administration. Additionally, these data support the fact that **2c** is at least twice as long acting as cocaine, and probably much more than this based upon casual observation wherein the animals treated with 0.8 mg/kg of **2c** were still hyperactive 24 h following administration.

In summation, compounds **2c** and **2d** bind with affinities which are 480–800-fold higher than that of cocaine, but unlike cocaine or, in part, compound **6**, they are not subject to the action of esterases. Consequently, as already found for **2c**, the duration of action of such compounds is expected to be longer than that of cocaine analogues which retain one or both ester bonds. Analogues like **2c** and **2d** may therefore find application in certain studies which require chronic drug treatment in order to achieve a measurable endpoint. Additionally, these analogues might also offer certain advantages in the preparation of radiolabeled ligands for use in PET or SPECT imaging studies.<sup>9</sup>

**Acknowledgment.** We are indebted to the National Institute on Drug Abuse for support of these studies.

**Supplementary Material Available:** Spectroscopic data for all new compounds reported herein and protocols for the drug discrimination studies (3 pages). Ordering information is given on any current masthead page.