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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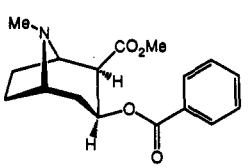
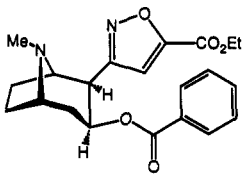
We have recently described efforts directed toward delineating structure-activity relationships for cocaine in an effort to identify a possible antagonist of cocaine's action at the dopamine re-uptake transporter.[1] During these efforts we discovered that this cocaine-recognition site is rather promiscuous 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 [^{3}H]mazindol binding and [^{3}H]dopamine uptake studies.



(-)-Cocaine



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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).[2] 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.[1] 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

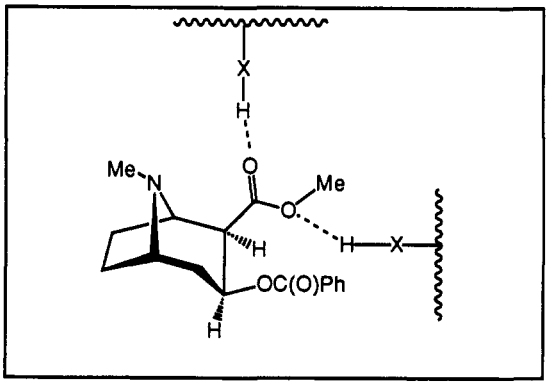
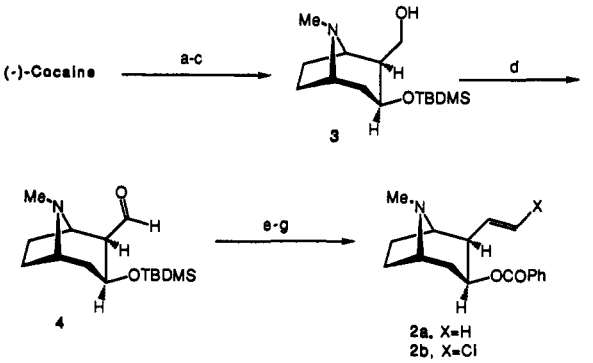


Figure 1. Illustration of possible hydrogen bonding to the C-2 ester group.

Scheme I [a]



[a] Reagents and conditions: (a) conc. H_2SO_4, MeOH, reflux, 18 h; (b) TBDMSCl, imd., rt, 20 h; (c) Dibal-H, CH_2Cl_2, -78 °C, 4 h; (d) Swern oxidation; (e) XCH=PPh_3; (f) F^-; (g) PhCOCl, pyr.

ester (**5**) as described by Carrol et al. in their preparation of additional analogues of the "Win" series of compounds.[3] The 2β-substituted isomer was separated from the 2α-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).[4]

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

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(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.

(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 3β-(*p*-Substituted phenyl)tropane-2β-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. 3β-Phenyltropane-2-carboxylic Esters and Analogs. *J. Med. Chem.* **1973**, *16*, 1260-1267.

(4) To ensure the β-stereochemistry of the 2-vinyl substituent of our analogues, the corresponding 2α-chlorovinyl isomer of **2d** was synthesized independently from the known 2α-ester. The ^{1}H NMR spectrum of the 2α-chlorovinyl isomer showed striking chemical shift differences from that of the 2β-isomer (e.g., =C(H)Cl appears at δ = 5.47 in the β-isomer and at 5.83 in the α-isomer).

(5) (a) Javitch, J. A.; Blaustein, R. O.; Snyder, S. H. [^{3}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**,

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Scheme II

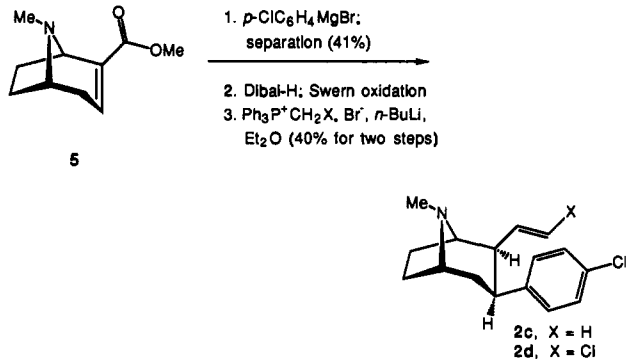

Table I. Dopamine Uptake and Mazindol Binding of Cocaine Analogues **2a–d** and **6**

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

endings (synaptosomes) in accordance with protocols described previously.[1,6] Mazindol was selected as the ligand to label the cocaine recognition site because of its known potency correlations with [^{3}H]dopamine uptake and reinforcing properties and similarities to cocaine binding.[5] These data are presented in Table I. Additionally, for comparison purposes, data are presented for (−)-cocaine and for 3β-(*p*-chlorophenyl)tropane-2β-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.[10] 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 (ED_{50}) was 1.7 mg/kg. Response rates were fairly stable across all test doses of cocaine. Testing at several intervals (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).

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[a] (min)	% drug responses[b]	rate[c] (responses/min)	*n*/*N*[d]
		Dose–Response Tests			
saline		15	9 ± 1	28 ± 4	7/7
cocaine	0.62	15	6 ± 2	27 ± 5	7/7
	1.25		44 ± 18	21 ± 5	7/7
	2.5		61 ± 16	32 ± 16	7/7
	5.0		98 ± 2	89 ± 16	7/7
	10.0		99 ± 1	76 ± 13	7/7
ED_{50} = 1.7 mg/kg					
		Temporal Tests			
cocaine	10.0	5	85 ± 13	28 ± 8	7/7
		15	99 ± 1	76 ± 13	7/7
		30	100	61 ± 11	7/7
		60	81 ± 14	23 ± 4	7/7
		120	31 ± 18	28 ± 7	7/7

[a] Time between drug injection and placement in operant chamber for the start of the substitution test. [b] Mean percentage of cocaine-appropriate responses (±SEM) during the test session. [c] Mean number of responses per min (±SEM) prior to completion of the first FR 20 observed during the test session. [d] *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[a]

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

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

Administration of **2c** (0.05–0.8 mg/kg) engendered a dose-dependent substitution for the cocaine cue (Table III). The 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).

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

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(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.; Ogren, 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 D_1 and 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 β-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 3β-phenyltropane-2β-carboxylic acid methyl ester and its para-substituted phenyl derivatives are the most potent cocaine analogues reported to date.[3] 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 3β-(*p*-fluorophenyl)tropane-2β-carboxylic acid methyl ester binding from rat striatal membranes.[3]

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^{-1} to the free energy of interaction of cocaine with its recognition site,[7] 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).[8] 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.[9]

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.