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EFFECTS OF VARIOUS ISOQUINOLINE ALKALOIDS ON IN VITRO ^{3}H-DOPAMINE UPTAKE BY RAT STRIATAL SYNAPTOSOMES

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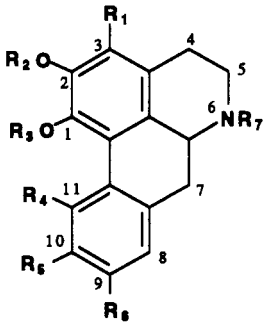
ABSTRACT.—Various alkaloids having an isoquinoline skeleton from different species of the Annonaceae, Fumariaceae, and Aristolochiaceae (aporphine, cularine, benzylisoquinoline, and bisbenzylisoquinoline derivatives) were tested for their ability to inhibit in vitro ^{3}H-dopamine uptake by rat striatal synaptosomes which was compared to their activity at striatal dopamine D_1 ^{3}H-SCH 23390 and D_2 ^{3}H-raclopride binding sites. Except for some aporphine derivatives (anonaine [**1**], norstephalagine [**2**], isopiline [**3**]) and some bisbenzylisoquinoline alkaloids (dimethylgrisabine [**27**], antioquine [**28**], obaberine [**29**], isotetrandrine [**30**])that displayed affinities of the same order as reference compounds (nomifensine [**38**], amineptine [**39**], dexamphetamine [**40**]), the other tested products had low, or no, affinity on ^{3}H-dopamine uptake. Only anonaine [**1**] appeared to display a good selectivity for ^{3}H-dopamine uptake since, in comparison, its affinity at dopamine D_1 ^{3}H-SCH 23390 and D_2 ^{3}H-raclopride binding sites was low. These data suggest that it could be possible to synthesize anonaine-like products displaying intense dopamine-uptake inhibitory properties, which could lead to a potential antidepressant activity.

Dopamine is a neurotransmitter in certain brain regions, namely, the striatum and the limbic system. Thus, dopamine is synthesized in specific neurons and is released in dopaminergic synaptic clefts to elicit physiological effects after binding at dopamine receptors. Dopamine receptors were originally divided into two subtypes, namely, the D_1 and D_2 types (1). More recent studies have reported the existence of multiple dopamine receptor types which can be grouped according to their structure and function into "D_1-like" and "D_2-like" dopamine receptor families (2). In the striatum, the affinity of dopamine agonists or antagonists for dopamine receptors can be determined by competition experiments using ^{3}H-SCH 23390 and ^{3}H-raclopride as selective ligands at D_1 (or "D_1-like") and D_2 (or "D_2-like") dopamine receptors, respectively (3,4).

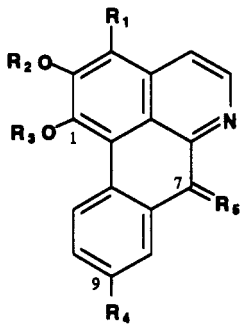
After its action at dopamine receptors, dopamine is taken out of the synaptic cleft by rapid uptake (or reuptake) by the dopaminergic nerve terminals. The uptake mechanism is achieved by membrane dopamine transporters of dopaminergic nerve endings (5) and leads to the replenishment of the releasable dopamine stores of the nerve terminals and to the enzymatic metabolism of dopamine. Various compounds are currently available as dopamine uptake inhibitors. These are natural products or synthetic compounds with a tropane skeleton such as cocaine (6), with an isoquinoline nucleus such as nomifensine [**38**] (7), with a tricyclic structure such as amineptine [**39**] (8), or with an arylpiperazine structure such as GBR 12783 (9). Their action on dopamine transporters increases dopamine concentration in the synaptic cleft and enhances dopaminergic neurotransmission. Such an effect supports the antidepressant activity of nomifensine [**38**] and of amineptine [**39**] (7,8).

In previous studies, it was observed that some isoquinoline alkaloids display a high affinity for D_1 and/or D_2 dopamine receptors. This is especially the case for aporphine derivatives (10), cularine derivatives (11), bisbenzylisoquinoline derivatives (12), and

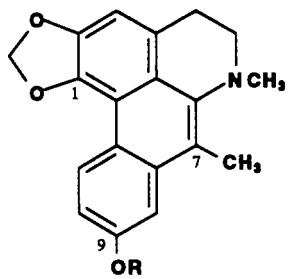
tetrahydroprotoberberine derivatives (13). From the well-characterized activity of nomifensine [**38**] as a dopamine uptake inhibitor (7) and from our previous data on the affinity of some isoquinoline alkaloids for dopamine receptors (11–13), we decided to test whether some natural isoquinoline alkaloids could act as dopamine uptake inhibitors. This study was carried out in vitro using ^{3}H-dopamine as a substrate for the dopamine transporters of synaptosomes (8,9,13). We have further determined if the tested products display a selectivity for dopamine transporters or for D_1 or D_2 dopamine receptors.



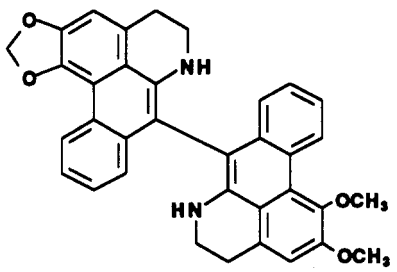
1 $R_1=R_4=R_5=R_6=R_7=H$; $R_2+R_3=-CH_2-$
2 $R_1=OCH_3$; $R_2+R_3=-CH_2-$; $R_4=R_5=R_6=R_7=H$
3 $R_1=OCH_3$; $R_2=CH_3$; $R_3=R_4=R_5=R_6=R_7=H$
4 $R_1=R_4=R_5=R_7=H$; $R_2+R_3=-CH_2-$; $R_6=OH$
5 $R_1=R_2=R_4=R_7=H$; $R_3=CH_3$; $R_5=OCH_3$; $R_6=OH$
8 $R_1=R_5=R_6=H$; $R_2+R_3=-CH_2-$; $R_4=OCH_3$; $R_7=CH_3$
9 $R_1=OCH_3$; $R_2=R_7=CH_3$; $R_3=R_4=R_5=R_6=H$
12 $R_1=R_6=H$; $R_2=R_3=R_7=CH_3$; $R_4=R_5=OH$



6 $R_1=OCH_3$; $R_2=CH_3$; $R_3=H$; $R_4=OH$; $R_5=(CH_3)_2$
7 $R_1=R_4=H$; $R_2+R_3=-CH_2-$; $R_5=O$
13 $R_1=R_4=H$; $R_2=R_3=CH_3$; $R_5=O$



10 $R=CH_3$
11 $R=H$



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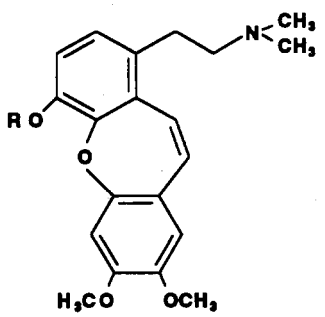
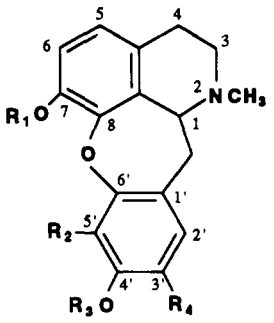
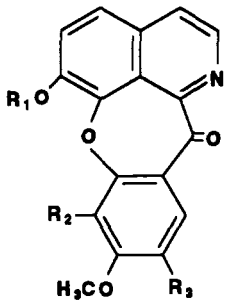


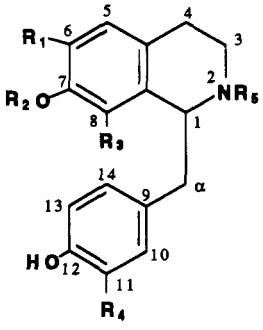
15 $R=H$
16 $R=CH_3$



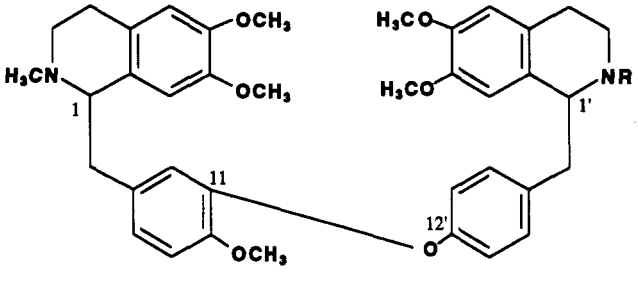


17 $R_1=R_2=H$; $R_3=CH_3$; $R_4=OCH_3$
18 $R_1=R_2=H$; $R_3=CH_3$; $R_4=OH$
19 $R_1=R_3=CH_3$; $R_2=OH$; $R_4=H$
20 $R_1=R_4=H$; $R_2=OH$; $R_3=CH_3$
21 $R_1=R_2=R_3=H$; $R_4=OCH_3$

22 $R_1=CH_3$; $R_2=H$; $R_3=OCH_3$
23 $R_1=R_3=H$; $R_2=OCH_3$



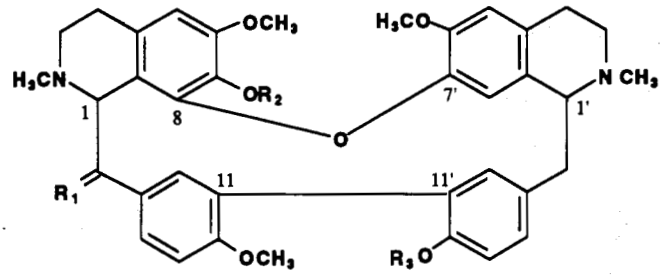
24 $R_1=OCH_3$; $R_2=R_3=R_4=R_5=H$
25 $R_1=H$; $R_2=R_5=CH_3$; $R_3=OH$; $R_4=OCH_3$
26 $R_1=OCH_3$; $R_2=CH_3$; $R_3=R_4=R_5=H$



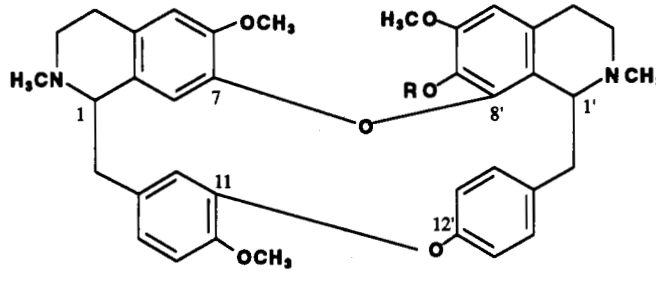
27 R=CH_3 (1*S*,1′*R*)
31 R=CH_3 (1*R*,1′*R*)
34 R=H (1*R*,1′*R*)

RESULTS AND DISCUSSION

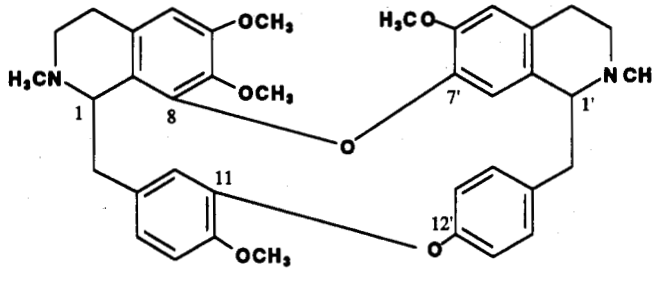
Most of the tested products (Table 1) appeared to be weak dopamine uptake inhibitors. This was especially true for cularine derivatives, among which some products (celtisine [**21**], cularidine [**17**], breoganine [**18**]) were described previously as very potent at D_1 and D_2 dopamine receptors (11) (Table 2). This was also the case for the benzylisoquinoline alkaloids tested which appeared at least 70 times less potent than *nomifensine* [**38**] as dopamine-uptake inhibitors (Tables 3 and 4). The products which



28 $R_1=R_2=R_3=H$ (1*S*,1′*R*)
33 $R_1=O$; $R_2=R_3=CH_3$ (1*S*,1′*R*)



29 $R=CH_3$ (1*R*,1′*S*)
32 $R=H$ (1*R*,1′*S*)



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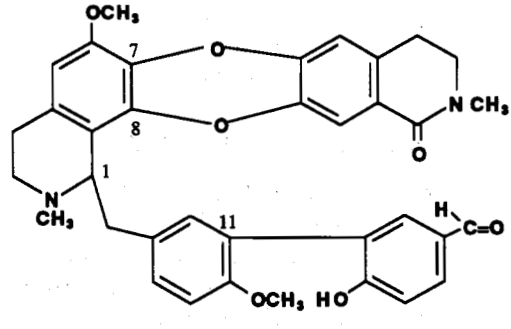

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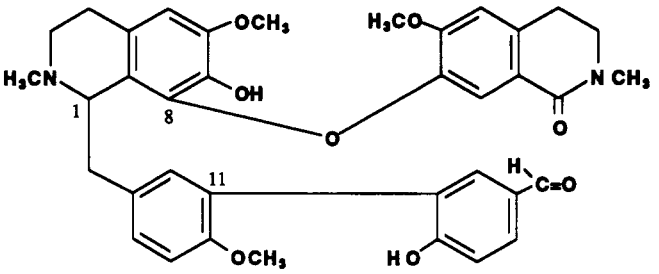

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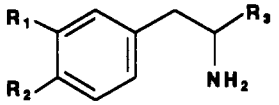
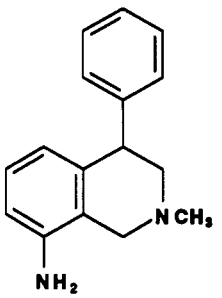


37 $R_1=R_2=OH$; $R_3=H$
40 $R_1=R_2=H$; $R_3=CH_3$



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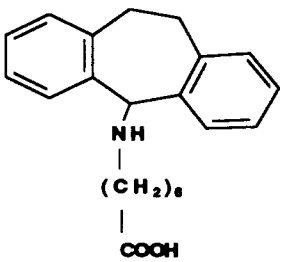

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were found to inhibit ^{3}H-dopamine uptake by rat striatal synaptosomes at concentrations roughly similar to the reference compounds were some of the aporphine derivatives and the bisbenzylisoquinoline derivatives represented. In the group of aporphinoid alkaloids, it appeared that the 6a,7-dehydroaporphines (belemine [**11**], *O*-methylbelemine [**10**]), the oxoaporphines (liriodenine [**7**], lysicamine [**13**]), and the dimeric derivative, heteropsine [**14**], were virtually devoid of activity as dopamine-uptake inhibitors. These products were also weakly effective for the displacement of ^{3}H-SCH 23390 and of ^{3}H-raclopride from dopamine D_1 and D_2 receptors in the striatum. The more active products on ^{3}H-dopamine uptake in the group of aporphine derivatives were anonaine [**1**] (Figure 1), norstephalagine [**2**], and isopiline [**3**] (Table 2). Furthermore, these compounds, especially anonaine [**1**], appeared to display an interesting selectivity as dopamine uptake inhibitors when their efficacy at dopamine D_1 and D_2 receptor binding sites was compared. From the chemical structures of the tested aporphines, it appeared that the presence of a group at position C-9 decreased the activity of aporphines as dopamine-uptake inhibitors. Furthermore, the presence of a methylenedioxy group, in place of hydroxy or methoxy groups at positions C-1 or C-2, appeared to lead to more active compounds. These data for the selectivity at the dopamine uptake sites contrast with the well-known structures of aporphines active at dopamine receptors (10). A comparison of the structures of cularines and aporphines suggested that the low activity of cularines as dopamine-uptake inhibitors could be due to the fact that, in addition to possessing a different skeleton from the aporphines, all the cularines tested had hydroxy or methoxy groups in positions C-3′, C-4′, and/or C-5′, and none had a C-6, C-7 methylenedioxy group. This could also be the reason for the inactivity of the tetrahydroprotoberberines previously studied as dopamine-uptake inhibitors (13).

In the case of the bisbenzylisoquinoline alkaloids, contrary to their efficacy at dopamine receptors (12), an obvious relationship between chemical structure and activity as dopamine-uptake inhibitors did not appear since compounds with roughly

TABLE 1. Origin of the Test Compounds Used in This Investigation.

Code	Compound	Plant from which isolated
Aporphine Derivatives		
1	Anonaine	*Artabotrys maingayi*
2	Norstephalagine	*Artabotrys maingayi*
3	Isopiline	*Guatteria ouregou*
4	Anolobine	*Guatteria schomburgkiana*
5	Laurolitsine (=Norboldine)	*Artabotrys maingayi*
6	Melosmine	*Guatteria ouregou*
7	Liriodenine	*Guatteria schomburgkiana*
8	*N*-Methylputerine (=*O*-Methylpukateine)	*Guatteria schomburgkiana*
9	*N*-Methyisopiline	*Guatteria ouregou*
10	*O*-Methylbelemine	Semi-synthetic
11	Belemine	*Guatteria schomburgkiana*
12	Suaveoline	*Artabotrys maingayi*
13	Lysicamine	*Guatteria ouregou*
14	Heteropsine	*Unonopsis pacifica*
Cularine derivatives		
15	Secocularidine	*Sarcocapnos crassifolia*
16	Secocularine	*Sarcocapnos saetavensis*
17	Cularidine	*Sarcocapnos crassifolia*
18	Breoganine	*Sarcocapnos crassifolia*
19	Sarcocapnidine	*Sarcocapnos saetavensis*
20	Claviculine	*Sarcocapnos crassifolia*
21	Celtisine	*Sarcocapnos crassifolia*
22	Oxocularine	*Sarcocapnos saetavensis*
23	Oxosarcophylline	*Sarcocapnos crassifolia*
Benzylisoquinoline and bisbenzylisoquinoline derivatives		
24	Coclaurine	*Artabotrys maingayi*
25	Isocrassifoline	Semi-synthetic
26	Norarmepavine	*Artabotrys maingayi*
27	Dimethylgrisabine	*Aristolochia gigantea*
28	Antioquine	*Pseudoxandra sclerocarpa*
29	Obaberine	*Pseudoxandra sclerocarpa*
30	Isotetrandrine	*Pseudoxandra sclerocarpa*
31	*O*-Methyldauricine	*Aristolochia gigantea*
32	Homoaromoline	*Pseudoxandra sclerocarpa*
33	Dimethylpseudoxandrine	Semi-synthetic
34	Pampulhamine	*Aristolochia gigantea*
35	Secolucidine	*Pseudoxandra sclerocarpa*
36	Secantioquine	*Pseudoxandra sclerocarpa*
Reference compounds		
37	Dopamine	
38	Nomifensine	
39	Amineptine	
40	Dexamphetamine	

similar activities were found in the groups of derivatives with one ether bridge at C-11, C-12′ (dimethylgrisabine [**27**]) and with two ether bridges at C-7, C-8′ or C-8, C-7′ and C-11, C-12′ (isotetrandrine [**30**], obaberine [**29**]), and with one ether bridge and one biphenyl bridge at C-8, C-7′ and C-11, C-11′ (antioquine [**28**]) (Table 3). However, seco derivatives were totally ineffective.

In conclusion, our data suggest that some structural modifications of the studied isoquinoline skeletons, and especially of anonaine-like structures, could lead to synthetic compounds with a high activity as dopamine uptake inhibitors. By comparison with the

TABLE 2. IC_{50} Values of Various Cularine Derivatives on ^{3}H-Dopamine Uptake by Rat Striatal Synaptosomes and Ratios of IC_{50} Values at ^{3}H-SCH 23390 D_1 Binding Sites and ^{3}H-Raclopride D_2 Binding Sites to IC_{50} Values on ^{3}H-Dopamine Uptake.[a]

Alkaloid	IC_{50} (μM) value on ^{3}H-dopamine uptake	Ratio of IC_{50} values	
		D_1/uptake	D_2/uptake
Secocularidine [**15**]	13.4 (2.9–62.2)	3.8	0.7
Secocularine [**16**]	28.2 (8.1–97.8)	>3.5	0.8
Cularidine [**17**]	41.2 (5.8–295.1)	0.002	0.008
Breoganine [**18**]	56.5 (8.1–395.3)	0.002	0.004
Sarcocapnidine [**19**]	85.5 (11.5–634.9)	0.58	0.023
Claviculine [**20**]	>100	<0.010	<0.015
Celtisine [**21**]	>100	<0.0006	<0.0003
Oxocularine [**22**]	>100	N.C.[b]	N.C.[b]
Oxosarcophylline [**23**]	>100	<0.6	N.C.[b]

[a]IC_{50} values on ^{3}H-dopamine uptake were calculated by the method of Litchfield and Wilcoxon (23) from concentration-effect curves with 3–5 concentrations and 6 determinations for each concentration. IC_{50} values at D_1 ^{3}H-SCH 23390 binding sites and at D_2 ^{3}H-raclopride binding sites were similarly calculated from concentration effect curves with 6–11 concentrations and 4 to 8 determinations for each concentration.
[b]Not calculable.

TABLE 3. IC_{50} Values of Various Benzylisoquinoline and Bisbenzylisoquinoline Derivatives on ^{3}H-Dopamine Uptake by Rat Striatal Synaptosomes and Ratios of IC_{50} Values at ^{3}H-SCH 23390 D_1 Binding Sites (D_1/uptake) and ^{3}H-Raclopride D_2 Binding Sites (D_2/uptake) to IC_{50} Values on ^{3}H-Dopamine Uptake.[a]

Alkaloid	IC_{50} value (μM) on ^{3}H-dopamine uptake	Ratio of IC_{50} values	
		D_1/uptake	D_2/uptake
Benzylisoquinoline derivatives			
Coclaurine [**24**]	14.1 (2.4–83.9)	0.35	0.03
Isocrassifoline [**25**]	36.2 (7.6–172.5)	0.18	0.27
Norarmepavine [**26**]	37.0 (13.9–98.5)	0.60	0.30
Bisbenzylisoquinoline derivatives			
Dimethylgrisabine [**27**]	1.1 (0.3–4.5)	5.2	1.3
Antioquine [**28**]	3.4 (0.8–14.1)	29.4	0.9
Obaberine [**29**]	3.9 (1.1–13.1)	10.0	7.3
Isotetrandrine [**30**]	4.2 (1.1–15.7)	7.9	0.15
O-Methyldauricine [**31**]	5.8 (1.2–26.9)	>17.2	>17.2
Homoaromoline [**32**]	10.9 (2.2–54.4)	>1.4	6.1
Dimethylpseudoxandrine [**33**]	20.1 (7.3–59)	1.1	0.19
Pampulhamine [**34**]	84.2 (38.6–183.6)	>1.2	>1.2
Secolucidine [**35**]	>100	N.C.[b]	<0.8
Secantioquine [**36**]	>100	N.C.[b]	<0.1

[a]IC_{50} values on ^{3}H-dopamine uptake were calculated by the method of Litchfield and Wilcoxon (23) from concentration-effect curves with 3–5 concentrations and 6 determinations for each concentration. IC_{50} values at D_1 ^{3}H-SCH 23390 binding sites and at D_2 ^{3}H-raclopride binding sites were similarly calculated from concentration effect curves with 6–11 concentrations and 4 to 8 determinations for each concentration.
[b]Not calculable.

well-known antidepressant activity of nomifensine (7), such synthetic compounds could display a similar therapeutic profile.

EXPERIMENTAL

PLANT MATERIAL.—Aporphine and benzylisoquinoline alkaloids were isolated from the leaves and bark

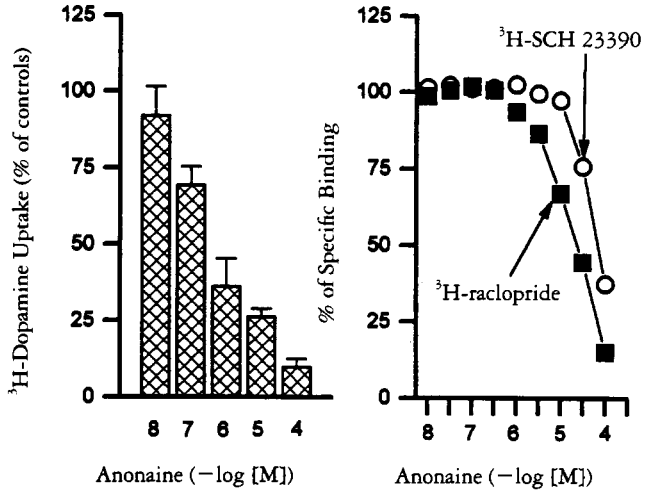

FIGURE 1. Comparative inhibition of ^{3}H-dopamine uptake and of specific ^{3}H-SCH 23390 and ^{3}H-raclopride binding by increasing concentrations of anonaine [**1**]. Values for ^{3}H-dopamine uptake are means±S.E.M. from 6–12 determinations; competition binding curves correspond to 4 values at each concentration.

TABLE 4. IC_{50} Values of Various Aporphine Derivatives on ^{3}H-Dopamine Uptake by Rat Striatal Synaptosomes and Ratios of IC_{50} Values at ^{3}H-SCH 23390 D_1 Binding Sites and ^{3}H-Raclopride D_2 Binding Sites to IC_{50} Values on ^{3}H-Dopamine Uptake.[a]

Alkaloid/Control Compound	IC_{50} value (μM) on ^{3}H-dopamine uptake	Ratio of IC_{50} values	
		D_1/uptake	D_2/uptake
Aporphine derivatives			
Anonaine [**1**]	0.8 (0.09–6.9)	85.0	23.5
Norstephalagine [**2**]	1.4 (0.08–24.9)	3.4	19.6
Isopiline [**3**]	2.5 (0.5–13.6)	3.9	13.6
Anolobine [**4**]	8.1 (0.9–72.1)	4.4	1.8
Laurolitsine [**5**]	16.5 (2.7–101.8)	0.15	0.41
Melosmine [**6**]	23.6 (4.5–123.3)	0.53	3.44
Liriodenine [**7**]	31.2 (12.3–79.2)	2.4	1.9
N-Methylputerine [**8**]	31.8 (4.1–243.8)	>3.1	>3.1
N-Methylisopiline [**9**]	46.4 (11.5–187.4)	0.37	0.06
O-Methylbelemine [**10**]	46.6 (7.7–281.7)	>2.1	>2.1
Belemine [**11**]	61.4 (5.8–648.2)	>1.6	>1.6
Suaveoline [**12**]	>100	<0.63	<0.005
Lysicamine [**13**]	>100	N.C.[b]	N.C.[b]
Heteropsine [**14**]	>100	N.C.[b]	N.C.[b]
Reference compounds			
Dopamine [**37**]	0.2 (0.05–1.2)	4.8	4.0
Nomifensine [**38**]	0.2 (0.03–1.5)	64	274
Amineptine [**39**]	1.0 (0.1–7.9)	>100	>100
Dexamphetamine [**40**]	4.9 (2.0–12.2)	>20.4	>20.4

[a]IC_{50} values on ^{3}H-dopamine uptake were calculated by the method of Litchfield and Wilcoxon (23) from concentration-effect curves with 3–5 concentrations and 6 determinations for each concentration. IC_{50} values at D_1 ^{3}H-SCH 23390 binding sites and at D_2 ^{3}H-raclopride binding sites were similarly calculated from concentration effect curves with 6–11 concentrations and 4 to 8 determinations for each concentration.

[b]Not calculable.

of *Guatteria ouregou* Dun (14), *Guatteria schomburgkiana* Mart. (15), *Artabotrys maingayi* Hk. f. & Th. (16), and *Unonopsis pacifica* R.E. Fries (17) (Annonaceae). Cularine alkaloids were isolated from the leaves of *Sarcocapnos crassifolia* (Desf.) DC. (18) and *Sarcocapnos saetavensis* Mateo et Figuerola (19) (Fumariaceae). Bisbenzylisoquinoline alkaloids were isolated from the bark of *Pseudoxandra sclerocarpa* Maas (Annonaceae) (20) and that of *Aristolochia gigantea* Mart. (Aristolochiaceae) (21). Voucher specimens are deposited as follows: *Pseudoxandra esclerocarpa* at the University of Antioquia, Medellin, Colombia and at the Museum d'Histoire Naturelle, Paris, France; *Aristolochia gigantea* at the Faculty of Pharmacy of the U.F.M.G., Belo Horizonte, Brazil; *Sarcocapnos crassifolia* at the Herbarium of the Department of Botany, University of Granada, Spain, *Sarcocapnos saetavensis*, Ref. 16001, at the Herbarium of the Department of Botany, University of Valencia, Spain, *Unonopsis pacifica*, Ref. J. Brand 1235, at the Herbarium of the Botanic Garden Joaquin Antonio Uribe, Medellin, Colombia; *Artabotrys maingayi*, Ref. K.L. No. 3408, at the Museum of the University of Malaya, Kuala Lumpur, Malaysia; *Guatteria schomburgkiana* at the Herbarium of the Faculty of Pharmacy, University of Belo Horizonte, Brazil; *Guatteria ouregou*, Ref. Moretti 1085, Centre ORSTOM, Cayenne, Guyane Française. Semi-synthesized products were obtained as described previously (15,18,20,22). A summary of these alkaloid isolations is provided in Table 1.

EXTRACTION AND ISOLATION.—The dried, powdered plant (usually stem bark; stems and leaves for *Sarcocapnos*) was defatted with petroleum ether. The marc was made alkaline with NH_4OH and extracted with CH_2Cl_2. The concentrated solution was extracted with HCl and the aqueous layer was made alkaline and extracted again with CH_2Cl_2, which was removed *in vacuo* to afford the crude alkaloids. Total alkaloid extracts were fractionated by flash chromatography followed by Si gel 60 H cc separations, to afford the isolated alkaloids (14–21). The identities of these alkaloids were determined by spectroscopic methods (ir, uv, ms, ^{1}H-nmr for all the products; ^{13}C-nmr, homonuclear correlations, ^{1}H-^{1}H COSY 45, and NOEDIFF, and heteronuclear correlations, ^{1}H-^{13}C XH CORR, for bisbenzylisoquinoline alkaloids like antioquine [**28**] and dimethylpseudoxandrine [**33**]).

^{3}H-DOPAMINE UPTAKE.—Male Wistar rats (150–250 g, IFFA-CREDO, L'Arbresle, France) were used throughout. All experimental procedures for the preparation of tissues were carried out at 0–4°. For the preparation of synaptosomes, rats were killed by decapitation and the striatum was dissected and homogenized in 10 volumes (w/v) of 0.32 M sucrose using 10 up-and-down strokes of a teflon glass homogenizer (800 rpm). Nuclear material was removed by centrifugation at 1000×*g* for 10 min. The supernatant (S1) was stored and the P1 pellet was resuspended in 10 volumes of 0.32 M sucrose and recentrifuged (1000×*g*) for 10 min. The two supernatants were combined and the mixture centrifuged at 15,000×*g* for 30 min. The resultant P2 pellet was suspended in 20 volumes of ice-cold Krebs-Ringer medium previously oxygenated (95% O_2–5% CO_2). The medium contained (mM): NaCl=109, KCl=3.6, KH_2PO_4=1.1, $CaCl_2$=2.4, $MgSO_4$=0.6, $NaHCO_3$=25, glucose =5.5; pH=7.6. ^{3}H-Dopamine uptake was evaluated on aliquots of the synaptosomal preparation. After a 5 min preincubation in Krebs-Ringer buffer containing 10 μM pargyline, ^{3}H-dopamine (47 Ci/mmole, Amersham, France) was added to a final 2 nM concentration. Five-minute incubations were stopped by dilution into ice-cold Krebs-Ringer medium followed by filtration *in vacuo* on Whatman GF/B filters. Filters were washed twice with 3 ml cold Krebs-Ringer medium and dried. Tissue radioactivity retained by synaptosomes was determined by liquid scintillation spectrometry. Blank values, obtained by incubating parallel samples at 0°, were subtracted (13).

COMPETITION EXPERIMENTS.—The displacement activity of the alkaloids on ^{3}H-raclopride and on ^{3}H-SCH 23390 binding sites in rat striatum was tested as described previously (2–4). Briefly, each striatum was homogenized in 2 ml ice-cold Tris-HCl buffer (50 mM, pH 7.4 at 22°) with a polytron (4s, maximal scale) and immediately diluted with Tris buffer. The homogenate was centrifuged (20,000×*g*, 10 min, 4°), then the pellet was washed by resuspension in the same volume of Tris buffer. For ^{3}H-SCH 23390 binding experiments, the final pellet was resuspended in Tris buffer containing 5 mM $MgSO_4$, 0.5 mM EDTA, and 0.02% ascorbic acid (Tris-Mg buffer). A 100-μl aliquot of membrane suspension (100 μg of striatal protein) was incubated for 1 h at 25° with 100 μl Tris-Mg buffer containing ^{3}H-SCH 23390 (85.5 Ci/mmol, New England Nuclear (NEN), Paris, France, 0.25 nM final concentration) and 800 μl of Tris buffer containing the compounds. Non-specific binding was determined in the presence of 30 μM SK&F 38393. For ^{3}H-raclopride binding experiments, the final pellet was resuspended in Tris buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid (Tris-ions buffer). A 200-μl aliquot of membrane suspension (200 μg of striatal protein) was incubated for 1 h at 25° with 200 μl of Tris-ions buffer containing ^{3}H-raclopride (86.5 Ci/mmol, NEN, Paris, France, 0.5 nM final concentration) and 400 μl of Tris-ions buffer containing the test compounds. Non-specific binding was determined in the presence of 50 μM apomorphine. In both cases, incubations were stopped by the addition of 3 ml of ice-cold buffer followed by rapid filtration through Whatman GF/B filters. Tubes were rinsed with 3 ml of ice-cold buffer and filters were washed with 3×3 ml ice-cold buffer. After the filters had been dried, radioactivity was counted in 4

ml BCS scintillation liquid (Amersham, Les Ulis, France) at an efficiency of 45%. This displacement activity was tested only for the products, the results of which were not previously published (11–13).

CALCULATIONS.—IC_{50} values and their 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon (23). In addition to IC_{50} values on ^{3}H-dopamine uptake, the selectivity of the tested products on ^{3}H-dopamine uptake or on ^{3}H-SCH 23390 or ^{3}H-raclopride binding was expressed from the ratios of IC_{50} value on ^{3}H-dopamine uptake/IC_{50} value on ^{3}H-SCH 23390 binding (=D_1/uptake) and IC_{50} value on ^{3}H-dopamine uptake/IC_{50} value on ^{3}H-raclopride binding (=D_2/uptake). When one of the IC_{50} values of the ratio was higher than 100 μM (highest tested concentration in all cases), the lower or higher limit of the ratio was indicated. The ratios could not be calculated when the two IC_{50} values were higher than 100 μM.

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