Displacement activity of some natural cularine alkaloids at striatal ^{3}H-SCH 23390 and ^{3}H-raclopride binding sites

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Abstract. Five natural cularines isolated from the aerial parts of *Sarcocapnos crassifolia* (Fumariaceae) and a cularioid isolated from the bark of *Guatteria ouregou* (Annonaceae) were tested for their ability to displace ^{3}H-SCH 23390 and ^{3}H-raclopride from their striatal binding sites. Celtisine, breoganine and cularidine were able to inhibit the binding at D-1 and D-2 dopaminergic sites at nanomolar concentrations. Other alkaloids were active at micromolar concentrations. These data suggest that the presence of an oxepine system in the isoquinoline skeleton could lead to compounds which would be very active and possibly selective at dopaminergic receptor sites.

Key words. Cularines; isoquinoline alkaloids; ^{3}H-SCH 23390; ^{3}H-raclopride; dopamine D-1/D-2 receptors.

The cularines are a group of isoquinoline alkaloids with a tetracyclic skeleton incorporating an oxepine system[1]. The occurrence of cularine alkaloids in nature appears to be associated with the Fumariaceae family[2]. Two cularioids have been isolated from the Annonaceae and Berberidaceae families[3,4]. The isolation of crassifoline, **1** (fig. 1), the first diphenolic 7,8,3',4'-tetraoxygenated tetrahydro-benzylisoquinoline from *Sarcocapnos crassifolia*[5], and the incorporation of radioactive crassoline into cularine, demonstrated the key role of crassifoline as the precursor of the biosynthesis of the cularine alkaloids[6]. Direct phenolic oxidative para or ortho coupling of crassifoline, **1**, would lead to cularine-type and isocularine-type alkaloids respectively. Recently, we have studied the effect of the cularine alkaloids on the Ca^{2+}-dependent contractile activity in an isolated rat uterus preparation[7]. The cularines displayed a relaxing activity on contractions induced by KCl and on rhythmic contractions induced by oxytocin in the presence of Ca^{2+}.

In this paper, we describe the displacing activity at central dopaminergic binding sites of five cularine alkaloids; (+)-cularidine, **2**, (+)-celtisine, **3**, (+)-breoganine, **4**, (+)-sarcocapnidine, **5**, (+)-claviculine, **6**; that of a cularinoid (gouregine, **7**,) and that of a benzylisoquinoline precursor of cularine alkaloids (isocrassifoline, **8**, fig. 1). We isolated these alkaloids from *Sarcocapnos crassifolia* (Fumariaceae)[5,8] except for gouregine, which was isolated from *Guatteria ouregou* (Annonaceae)[3]. The powdered aerial parts (*Sarcocapnos crassifolia*) or bark (*Guatteria ouregou*) were extracted with MeOH in a Soxhlet until a Mayer's test was negative. After removal of solvent, the residues were taken up in 10% HCl and filtered. The acidic extracts were washed with petrol to remove neutral compounds. The acidic solutions were extracted with CH_2Cl_2 after successive additions on NH_4OH. TLC showed that the crude extracts were very complex mixtures. They were purified on silica gel

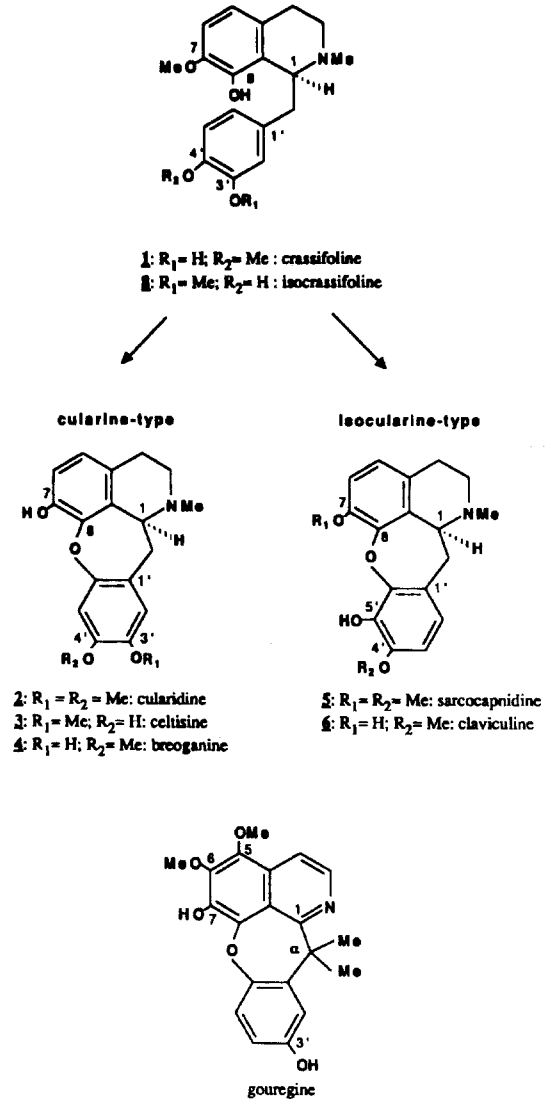

Figure 1. Chemical structure of cularine-type and isocularine-type alkaloids. Comparison with benzylisoquinoline precursors and with gouregine.

columns eluted with CH_2Cl_2 containing increasing proportions of MeOH[9]. The structure of the isolated cularine alkaloids was elucidated by spectroscopic methods, especially ^{1}H-NMR (see table 1).

Cularine derivatives were tested for their activity at two pharmacologically defined dopamine receptors, subtypes (D-1 and D-2)[10], by studying the displacement of specific binding of ^{3}H-SCH 23390, a selective ligand at D-1 dopamine receptors[11], and of ^{3}H-raclopride, a selective ligand at D-2 dopamine receptors[12]. Binding experiments were performed on striatal membranes from male Wistar rats (200–250 g, IFFA-CREDO, L'Arbresle, France). One striatum was homogenized in 2 ml ice-cold Tris-HCl buffer (50 mM, pH = 7.4 at 22 °C) with a polytron (4 s, maximal scale) and immediately diluted with Tris-buffer. The homogenate was centrifuged either twice (^{3}H-SCH 23390 binding experiments) or four times (^{3}H-raclopride binding experiments) at 20,000 g for 10 min at 4 °C (PR20, Jouan, Issy-les-Moulineaux, France) with resuspension in the same volume of Tris buffer between each centrifugation.

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) and the suspension was briefly sonicated, then diluted to a protein concentration of 1 mg/ml[13]. A 100-µl aliquot of freshly prepared membrane suspension (100 µl of striatal protein) was incubated for 1 h at 25 °C with 100 µl of Tris-Mg buffer containing ^{3}H-SCH 23390 (70.3 Ci/mmol, NEN, Paris, France, 0.25 nM final concentration) and 800 µl of Tris-Mg buffer containing the required drugs. Non-specific binding was determined in the presence of 30 µM SK&F 38393 (RBI, Natick, USA)[14], and represented around 2–3% of total binding.

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 $MgCl_2$, 0.1% ascorbic acid (Tris-ions buffer) and the suspension was treated as described above. A 200-µl aliquot of freshly prepared membrane suspension (200 µg of striatal protein) was incubated for 1 h at 25 °C with 200 µl of Tris-ions buffer containing ^{3}H-raclopride (75.6 Ci/mmol, NEN, Paris, France, 0.5 nM final concentration) and 400 µl of Tris-ions-buffer containing the drug being investigated. Non-specific binding was determined in the presence of 50 µM apomorphine[15], and represented around 5–7% of total binding.

In both cases, incubations were stopped by the addition of 3 ml ice-cold buffer (Tris-Mg buffer or Tris-ions buffer as appropriate) followed by rapid filtration through Whatman GF/B filters. Tubes were rinsed with 3 ml buffer and filters were washed with 3 × 3 ml buffer. After the filters had been dried, radioactivity was counted in 4 ml ACS II scintillation liquid (Amersham, Paris, France) at an efficiency of 45%. Filter blanks corresponded to approximately 0.5% of total binding and were not modified by drugs. All the compounds were tested comparatively (only 6 concentrations from 10^{-9} to 10^{-4} M) because only very low amounts (< 1 mg) of some products were available. A more detailed analysis was done for the most active compounds. IC_{50} values and their 95% confidence limits were calculated by the method of Lichtfield and Wilcoxon[16].

In these experimental conditions, IC_{50} values determined for reference compounds (SK&F 38393, dopamine and quinpirole) were roughly similar to those previously reported[17]. Some cularine derivatives (celtisine, **3**, breoganine, **4**, and cularidine, **2**) were effective for displacing both ^{3}H-SCH 23390 and ^{3}H-raclopride specifically bound at low concentrations (fig. 2; table 2). Isocrassifoline, **8**, and the other cularine derivatives (claviculine, **6**, gouregine, **7**, and sarcocapnidine, **5**) were less effective. None of the cularine derivatives appeared to display a strong preferential efficacy at one of the two subtypes of dopamine receptor, like SK&F 38393 at the D-1 receptor subtype or quinpirole at the D-2 receptor subtype[17]. The displacing activity of effective cularine alkaloids appeared to be comparable to that of dopamine. Computer analysis of displacement curves of striatal ^{3}H-SCH 23390 and ^{3}H-raclopride binding by celtisine, **3**, breoga-

Table 1. ^{1}H-NMR ($CDCl_3$, δ) of the cularine alkaloids from *S. crassifolia* and *G. ouregou* as drawn in fig. 1 with their corresponding numbers.

	Cularidine **2**	Celtisine **3**	Breoganine **4**	Sarcocapnidine **5**	Claviculine **6**	Gouregine **7**
H-1	4.25	4.27	4.24	4.48	4.47	–
H-3,4,α	2.65–3.18	2.76–3.16	2.77–3.13	2.73–3.50	2.76–3.51	8.16/7.69
H-5	6.79	6.78	6.79	6.91	6.77	–
H-6	6.79	6.78	6.79	6.74	6.77	–
H-2′	6.72	6.78	6.70	6.57	6.53	6.93
H-3′	–	–	–	6.57	6.53	–
H-4′	–	–	–	–	–	6.68
H-5′	6.56	6.49	6.61	–	–	7.10
NMe	2.54	2.55	2.54	2.59	2.58	–
$(Me)_2$-α	–	–	–	–	–	1.90
OMe-5	–	–	–	–	–	3.96
OMe-6	–	–	–	–	–	4.15
OMe-7	–	–	–	3.87	–	–
OMe-3′	3.83	3.77	–	–	–	–
OMe-4′	3.79	–	3.85	3.87	3.79	–

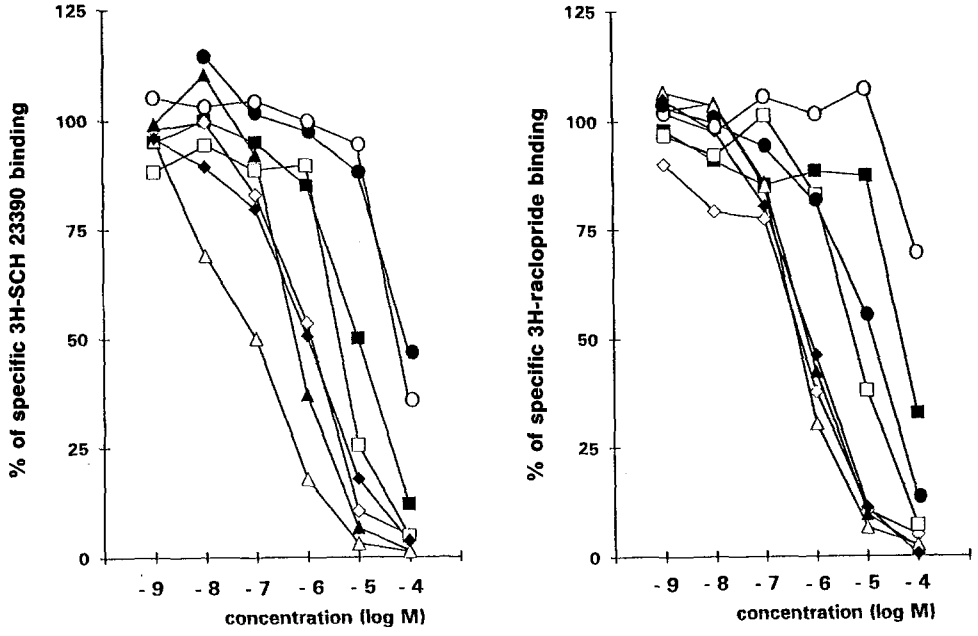

Figure 2. Inhibition of specific ^{3}H-SCH 23390 (left panel) and ^{3}H-raclopride (right panel) binding to rat striatal membranes by culariné and isocularine alkaloids, isocrassifoline and dopamine. Displacement curves correspond to values obtained from 2–3 experiments performed in duplicate. △——△ celtisine; ▲——▲ breoganine; ◇——◇ cularidine; ◆——◆ dopamine; □——□ claviculine; ■——■ isocrassifoline; ○——○ gouregine; ●——● sarcocapnidine.

Table 2. Comparative IC_{50} of culariné alkaloids and of isocrassifoline, SK&F 38393, dopamine and quinpirole for the displacement of specific ^{3}H-SCH 23390 and ^{3}H-raclopride binding to rat striatal membranes. IC_{50} and their 95% confidence limits were calculated from 2–6 experiments performed in duplicate.

Compounds	IC_{50} (μM) ^{3}H-SCH 23390	^{3}H-raclopride	Ratio D-1/D-2
SK&F 38393	0.03 (0.01–0.23)	3.81 (1.25–17.1)	0.008
Celtisine	0.06 (0.03–0.16)	0.03 (0.01–0.07)	2.00
Cularidine	0.08 (0.03–0.20)	0.32 (0.06–1.76)	0.25
Breoganine	0.10 (0.03–0.33)	0.22 (0.05–0.88)	0.45
Dopamine	0.96 (0.07–12.5)	0.79 (0.18–3.48)	1.22
Claviculine	1.04 (0.32–3.37)	1.55 (0.38–6.39)	0.67
Isocrassifoline	5.48 (1.09–27.5)	9.95 (1.83–54.1)	0.55
Sarcocapnidine	49.8 (6.6–262)	2.01 (0.59–6.81)	24.8
Gouregine	68.8 (15.4–307)	> 100	< 0.7
Quinpirole	> 100	0.22 (0.05–0.93)	> 455

nine, **4**, and cularidine, **2**, (fig. 3) indicated that they were best fitted using a two-site model, as for the displacement by dopamine agonists of binding at D-1 receptor[18] or at D-2 receptor sites[19].

Some conclusions can be drawn from the activities of culariné at dopaminergic binding sites. The comparison of the IC_{50} values of some of the tested compounds for binding to subtypes of striatal dopaminergic receptor sites, and for KCl-induced contraction of smooth muscle[7], indicated that isocrassifoline had roughly similar and relatively low effects on both systems. Cularidine, **2**, and celtisine, **3**, were about 70 and 1000 fold, respectively, more active as displacers of ^{3}H-SCH 23390 and ^{3}H-raclopride binding to rat striatal membranes. The presence of a hydroxy group in the 7 position appears to be very important for the displacement of ligands at D-1 dopaminergic binding sites. Indeed, compared to sarcocapnidine, **5**, which has a methoxy group in the 7 position, all other culariné or isocularine have a better displacing activity at D-1 dopaminergic binding sites. In contrast, the presence of a hydroxy group in the 5′ position of isocularine alkaloids appears to decrease their affinity for dopaminergic binding sites. In addition, the methylation or the blockade of hydroxy groups of the D ring led to a decreased affinity for D-2 binding sites. For example, celtisine, **3**, and breoganine, **4**, were more active than cularidine, **2**. These results contrast with the effects observed for culariné alkaloids on smooth muscle. The calcium antagonist activity was effectively increased when hydroxy groups were blocked[7].

As far as the activity of gouregine, **7**, is concerned, it appears that the oxidation of the pyridine ring, leading to less basic compounds, decreased the affinity for dopaminergic receptors as compared to classical culariné. Finally, the displacing activity of culariné at dopaminergic binding sites seems to be almost identical to that of aporphines, including apomorphine, a classical non-selective dopamine agonist[20]. However, the aporphine skeleton is more plane than that of culariné, in which the oxepine system leads to a molecular torsion that could modify the interactions at dopaminergic binding sites (fig. 4)[20].

In conclusion, our results indicate that some culariné alkaloids (celtisine, breoganine, cularidine), could be potential dopaminergic drugs in vivo and that the introduction of an oxepine system into the isoquinoline skeleton could generate compounds which would be very active and possibly selective at two classical subtypes of dopaminergic receptor sites.

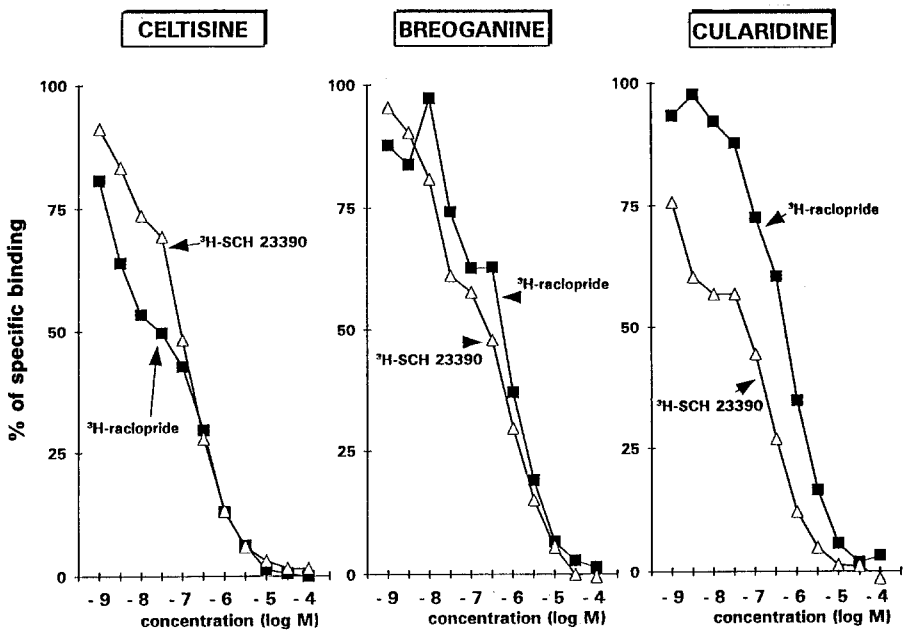

Figure 3. Comparative displacement curves of ^{3}H-SCH 23390 and ^{3}H-raclopride binding by celtisine, breoganine, and cularidine. Displacement curves correspond to values obtained from 2 experiments performed in duplicate.

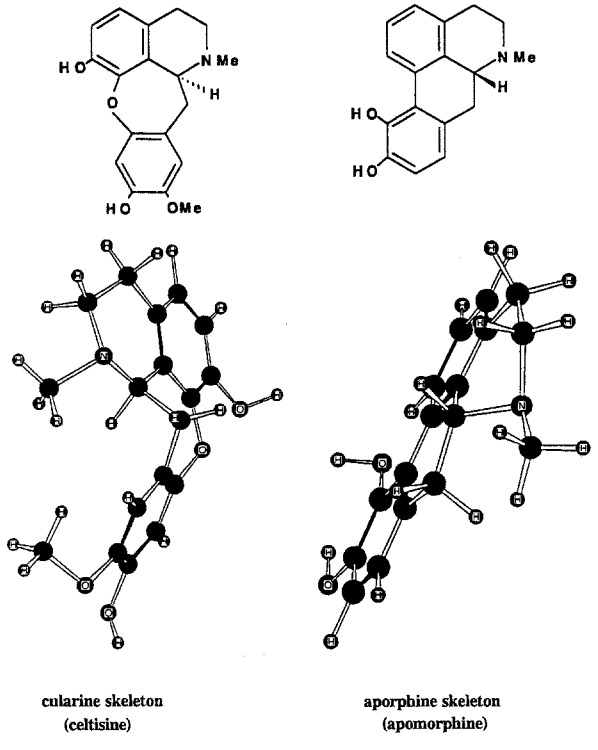

Figure 4. Chemical structures and tri-dimensional representations of celtisine and apomorphine (cularine and aporphine skeletons, respectively).

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