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Microbial Hydroxylation of Some Synthetic *Aristotelia* Alkaloids

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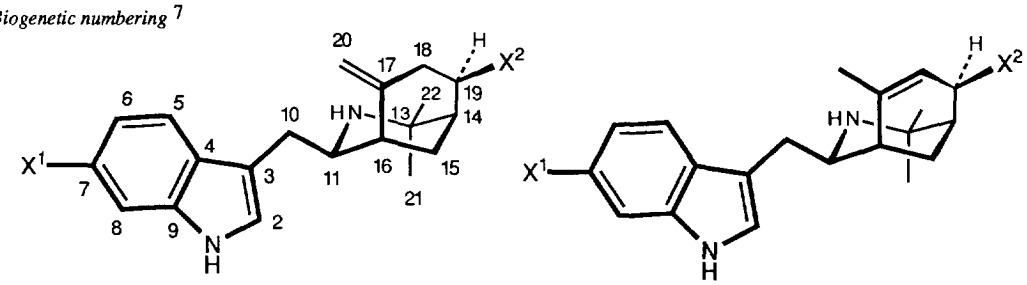
Abstract: Synthetically prepared, optically pure samples of the rare *Aristotelia* alkaloids (+)-makomakine (**1**), (–)-hobartine (**4**), and (+)-aristoteline (**7**), were exposed to twelve selected fungal strains and have been shown to afford, sometimes in preparatively acceptable yield, known, as well as hitherto unknown hydroxylated derivatives thereof.

The *Aristotelia* alkaloid family [1] comprises some 40 members that are closely interrelated biogenetically. The tetracyclic metabolites makomakine ((+)-**1**) [2] and hobartine ((–)-**4**) [3] *(Scheme 1)* are likely derived from tryptamine and an intact unrearranged monoterpene building block and conceivably serve as precursors for the lead alkaloid aristoteline ((+)-**7**) [4] *(Scheme 2)*. Most of the remaining representatives are situated on a higher oxidation level and many of them have been synthesized through slight modifications of the original strategy [5]. Nevertheless, some positions in the skeleton are not attainable this way; therefore, the microbial oxidation [6] of the readily available, synthetically prepared alkaloids (+)-**1**, (–)-**4**, and (+)-**7** was investigated. Out of the twelve commercially available strains tested (see Exper. Part), four were found to metabolize these alkaloids (see *Table 1* and *2*).

Scheme 1

Biogenetic numbering [7]



(+)-**1** $X^1 = X^2 = H$
(+)-**2** $X^1 = OH$, $X^2 = H$
(+)-**3** $X^1 = H$, $X^2 = OH$

(–)-**4** $X^1 = X^2 = H$
(–)-**5** $X^1 = OH$, $X^2 = H$
(–)-**6** $X^1 = H$, $X^2 = OH$

Table 1. Microbial Transformation of the Tetracyclic Alkaloids (+)-1 and (–)-4.

Start. mat.	Microorganism	Products [% isol. yield]					
		1	**2**	**3**	**4**	**5**	**6**
(+)-**1**	*Cunninghamella echinulata NRRL 3655*	12	51	7			
	Cunninghamella baineri ATCC 9244	–	34	6			
(–)-**4**	*Cunninghamella echinulata NRRL 3655*				25	23	30
	Cunninghamella baineri ATCC 9244				–	64	18
	Mucor plumbeus CBS 110-16				73	–	10

Two *Cunninghamella* strains reacted wih the tetracyclic alkaloids (+)-**1** and (–)-**4** in an analoguous fashion, since in both cases either position 7 or 19_{exo} was attacked to give the corresponding hydroxylated products (+)-**2** or (+)-**3**, and (–)-**5** or (–)-**6**, respectively. While the first three of these alkaloids were isolated for the first time, the racemic form of **6** had already been prepared before [8]. The localization of the phenolic hydroxyl group at C(7) followed from the ^{13}C-NMR spectra of (+)-**2** and (–)-**5** (see *Table 4:* characteristic shielding of the quaternary carbon C(4) in *para*-position to OH by 5.6 ppm as compared to the starting materials) and is in accordance with pertinent reference values [9]. In the case of (+)-**2** this structural assignment was corroborated by means of a positive NOE between the indolic *H*-N(1) and the isolated *H*-C(8) at 6.72 ppm which shows no *ortho*-coupling.

A byproduct in the oxidation of (+)-**1** with either of the two *Cunninghamella* strains was shown to be the hitherto unknown alkaloid 19_{exo}-hydroxymakomakine ((+)-**3**) by means of an analysis of its NMR spectra. Evidently, a hydrogen atom in one of the four methylene groups of the starting material has been replaced by a hydroxyl group (only three triplets left in the ^{13}C-NMR and appearance of an additional doublet at 68.8 ppm). Out of the four possible sites of attack, position 10 is ruled out, because the corresponding typical ABX-system is clearly discernible in the ^{1}H-NMR-spectrum of the product. Since the familiar pattern of the C(15)-methylene group is likewise still present, the remaining sites of attack are represented by positions 18 and 19. The former possibility is ruled out by the fact that the α-carbon C(17) is shielded by some 4 ppm as compared to the starting material; on the other hand, C(14) is deshielded by ca. 7 ppm, which clearly points to position 19 as the point of attachment of the incoming hydroxyl group. The axial nature of this substituent is evident from the *syn*-γ effect that it exerts on C(17) and C(15) (see *Table 4* and *Figure)* and from the marked deshielding of H_{anti}-C(15) in (+)-**3** as compared to (+)-**1** (see *Table 3).*

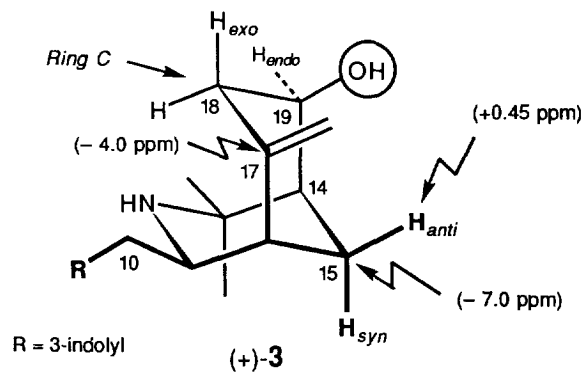
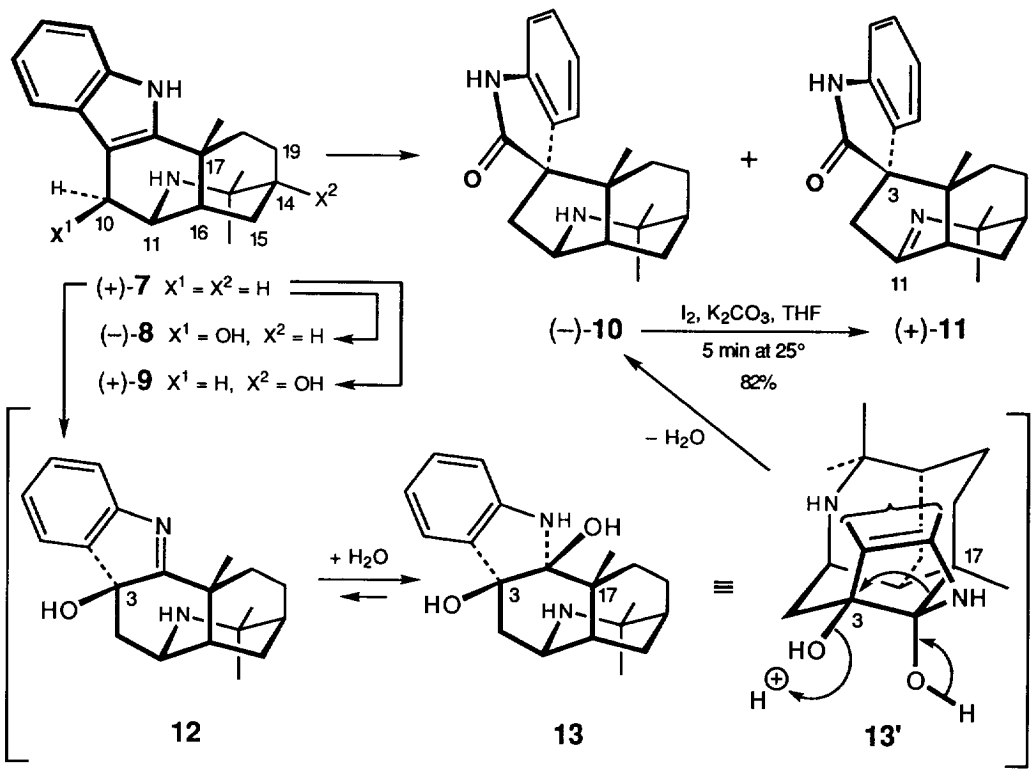


Figure. Some Significant Chemical-Shift Differences between (+)-3 and the Parent Compound (+)-1.

Somewhat more intriguing results were obtained when the pentacyclic representative (+)-aristoteline ((+)-**7**) *(Scheme 2)* was exposed to the same microorganisms. The three strains listed in *Table 2* led to interesting biotransformations of this alkaloid; *Mucor plumbeus CBS 110-16*, for instance, furnished four products of oxidation. Two of these, namely 10_{exo}-hydroxyaristoteline ((–)-**8**) [10] and (–)-tasmanine ((–)-**10**), a natural product isolated by others from *Aristotelia peduncularis* [11] and *A. serrata* [12], are known compounds that have been synthesized before [13].

Scheme 2



The two other products that were isolated from the above incubation mixture were unknown alkaloids. The highly characteristic spectroscopic data of the minor component readily pointed to structural proposal **11**. We verified this hypothesis by means of a partial synthesis of (+)-**11** from synthetic tasmanine ((–)-**10**), taking advantage of a recently developed, efficient method to dehydrogenate secondary amines to the corresponding imines [10].

Table 2. Microbial Transformations of Aristoteline ((+)-7).

Start. mat.	Microorganism	Products [% isol. yield]				
		7	**8**	**9**	**10**	**11**
(+)-**7**	*Mucor plumbeus CBS 110-16*	7	4	23	17	6
	Actinomucor elegans MMP 3122	50	–	17	–	–
	Cunninghamella baineri ATCC 9244	58	–	9	3	–
	Sterilized culture medium (blank)	>95	–	–	–	–

The major product, also produced by *Actinomucor elegans* and *Cunninghamella baineri*, had the composition $C_{20}H_{26}N_2O$. The nature of the newly introduced oxygen function was readily apparent in the ^{13}C-NMR spectrum: one of the three aliphatic doublets of the starting material (+)-**7** was missing and replaced by a singlet at 70.8 ppm. Of the two possible positions to place the tertiary hydroxyl group, C(14) or C(16), the latter was ruled out, because no deshielding of C(11) and C(17) as compared to (+)-**7** was noticed. On the other hand, the expected β-effect (4 to 9 ppm) is manifest in the chemical-shift values of C(13), C(15), and C(19) (see *Table 4*). Thus, this major product must be 14-hydroxyaristoteline ((+)-**9**) which has been unavailable so far through the established chemical *in vitro* strategies.

The reproducible transformation of aristoteline ((+)-**7**) into tasmanine ((–)-**10**) by *M. plumbeus* and *C. baineri* deserves some comment. A blank experiment and the unsuccessful incubations with the other tested strains clearly showed that the observed transformation is not an artifact, but the result of an enzymatically controlled oxidation reaction. Conceivably, the reaction sequence proceeds through 3-episerratoline (**12**) which may be formed *via* a 1*H*-indole-2,3-oxirane [15]. The alleged intermediate **12** was synthesized recently and shown to be very unstable; just on standing in $CDCl_3$ at 25°, for instance, it rearranges spontaneously and quantitatively to tasmanine ((–)-**10**), presumably through the hydrated intermediate **13** [13,16]. It seems as if *Aristotelia* plants contain two enzymes that are capable to oxidize position 3 of the aristoteline skeleton: one attacking from the more hindered concave side to produce the perfectly stable natural product serratoline (C(3)-epimer of **12**) [17], the other one acting from the convex face to yield tasmanine via the reactive intermediate **12** which does not survive the standard protocol that is generally employed to isolate and purify the alkaloids occuring in the *Aristotelia* plants.

Table 3. ^1H-NMR Chemical-Shift Values [ppm from TMS] in $CDCl_3$.

Cpd. / H	1	2	3	4	5	6[a]	7	8	9	10	11
H–C(2)	7.00	6.83	7.00	7.09	6.84	7.07	–	–	–	–	–
H–C(5)	7.63	7.43	7.62	7.64	7.38	7.62	7.45	7.64	7.44	7.39	7.27
H–C(6)	7.10	6.65	7.11	7.11	6.62	7.11	7.05	7.10	7.06	6.99	6.97
H–C(7)	7.18	–	7.19	7.18	–	7.20	7.11	7.15	7.12	7.17	7.19
H–C(8)	7.34	6.72	7.36	7.35	6.58	7.36	7.29	7.32	7.29	6.81	6.86
H–C(10)	2.76	2.70	2.77	2.82	2.75	2.76	3.07	4.67	3.07	2.55	3.13
H–C(10')	2.62	2.59	2.61	2.69	2.65	2.65	2.63	–	2.61	1.76	2.35
H–C(11)	3.49	3.45	3.45	3.49	3.49	2.85	3.62	3.54	3.63	3.76	–
H–C(14)	1.40	1.40	1.50	1.46	1.46	1.35	1.40	1.43	–	1.31	1.59
H_{syn}–C(15)	2.12	2.12	1.91	2.08	2.08	1.95	2.06	2.04	2.02	2.10	2.16
H_{anti}–C(15)	1.59	1.59	2.05	1.62	1.62	1.81	1.97	1.97	1.89	1.56	1.62
H–C(16)	2.27	2.26	2.28	2.17	2.19	2.20	1.70	1.88	2.00	2.53	3.24
H_{endo}–C(18)	3.08	2.98	3.46	5.63	5.64	5.80	2.62	2.32	2.44	3.03	1.94
H_{exo}–C(18)	2.18	2.19	2.26				1.61	1.54	1.77	0.75	0.82
H_{endo}–C(19)	2.07	2.07	4.25	2.28	2.28	4.27	1.92	1.88	2.02	1.94	1.87
H_{exo}–C(19)	1.49	1.50	–	2.08	2.07	–	1.67	1.65	1.65	1.53	1.49
H–C(20)	4.77 4.58	4.78 4.58	4.86 4.75	1.81	1.80	1.89	1.46	1.48	1.47	0.88	0.96
H_3–C(21)	1.14	1.16	1.17	1.16	1.20	1.16	1.29	1.30	1.32	1.20	1.29*
H_3–C(22)	1.10	1.12	1.08	1.09	1.12	1.15	0.97	1.04	1.06	1.14	1.38*

[a] Values taken from ref. 8.

* Assignments may be interchanged.

These results point out again the advantages of such microbial one-step transformations to easily obtain, from usual precursors, sizable amounts of rare, difficult-to-prepare, or yet unavailable alkaloid products.

Table 4. ^{13}C-NMR Chemical-Shift Values [ppm from TMS] in $CDCl_3$.

Cpd. / Carbon	1[a]	2	3	4[a]	5	6[c]	7[a]	8[b]	9	10[a]	11
2	122.3	121.2	122.3	122.3	121.0	122.2	142.6	145.8	141.8	184.5	183.0
3	113.9	113.6	113.6	113.5	113.4	113.4	104.4	106.1	104.5	62.9	58.7
4	128.0	122.4	127.8	127.6	122.0	127.4	128.2	127.4	128.1	131.8	130.2
5	119.3	119.8	119.2	118.9	119.4	119.3	118.2	118.2	118.1	126.7	125.7
6	119.1	109.3	119.2	118.9	109.5	119.0	119.1	119.6	119.2	121.8	121.9
7	121.8	152.1	121.9	121.6	152.3	122.0	121.0	121.5	121.2	127.8	128.0
8	110.0	97.0	111.0	111.0	97.1	111.1	110.5	110.9	110.5	109.2	109.3
9	136.5	137.3	136.4	136.3	137.3	136.4	136.1	136.6	136.1	141.7	141.6
10	31.5	31.3	31.5	31.7	31.6	31.7	28.6	66.7	28.1	44.3	41.2
11	54.2	54.2	53.9	54.6	54.6	53.5	50.4	58.4	49.9	53.6	173.9
13	53.1	53.2	51.7	54.2	54.0	51.7	53.3	53.1	57.4	54.0	56.6
14	35.9	36.6	43.3*	35.1	35.2	44.4	35.6	36.0	70.8	36.1	35.8
15	33.3	33.1	26.3	29.3	29.2	25.3	27.9	27.2	35.6	23.9	21.8
16	43.5	43.1	43.8*	38.3	38.2	39.2	39.3	34.8	44.6	41.5	40.9
17	150.6	150.1	146.6	133.5	133.5	139.1	33.2	33.3	32.7	48.2	43.6
18	32.0	32.0	40.5	124.7	124.9	126.7	36.0	36.0	37.9	32.3	30.3
19	29.4	29.2	68.8	27.9	27.9	65.8	25.5	25.2	34.0	25.9	25.7
20	108.6	109.1	111.8	25.7	25.6	25.7	25.2	24.8	25.0	19.8	16.8
21	27.2	27.0	27.3	25.9	25.7	26.1	27.6	27.1	23.0	27.7	29.0*
22	29.8	29.7	29.2	30.0	29.8	29.6	29.1	28.6	24.9	30.5	29.2*

[a] Assignments corroborated through HETCOR experiments performed with synthetically prepared material [13, 14].

[b] $CDCl_3 + CD_3OD$, ca. 1:1; values taken from ref. 10.

[c] Values taken from ref. 8.

* Assignments may be interchanged.

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EXPERIMENTAL PART

General. All solvents employed as reaction media were reagent grade *(Fluka, puriss.)* and were further purified and dried as follows: CH_2Cl_2, $CHCl_3$, and $CDCl_3$, filtered through Al_2O_3 *(Woelm,* basic act. I). M.p. (not corrected): *Tottoli* apparatus, sealed evacuated capillaries, unless mentioned otherwise. Optical rotations: *Perkin-Elmer 241.* UV spectra: *Uvikon 860.* IR spectra: *Perkin-Elmer 781.* ^{1}H-NMR spectra (δ [ppm] from TMS, apparent coupling constants *J* [Hz]): *Bruker AMX 400* (400 MHz) or *Bruker AMX 500* (500 MHz). ^{13}C-NMR spectra (in $CDCl_3$, δ [ppm] from TMS, multiplicities as determined from DEPT spectra): *Bruker AMX 400* (100 MHz). ^{1}H / ^{13}C-COSY (HETCOR) spectra were recorded on a *Varian Gemini* (300 / 75 MHz). Mass spectra (m/z [amu] (% base peak)): *VG TRIBID* (EI, 70 eV; for FAB: 3-nitrobenzyl alcohol as matrix) or GC-MS: *HP 5890 series II* GC with *HP 5971A* MS, EI (70 eV) T= 190°; column: *Supelco SPB-5*, fused silica, 30 m, Ø 0.25 mm, film thickness 0.25 μm; mode: 25-90°: 70°/min., 90-300°: 10°/min., hold at 300°: 30 min.

Starting materials. (+)-**1** $[\alpha]_D^{20}$ = + 145 (c 0.92, $CHCl_3$): prepared according to a modified version [16,19] of the original protocol of *Stevens* and *Kenney* [2b]. (–)-**4** $[\alpha]_D^{20}$ = - 28 (c 0.92, $CHCl_3$): synthesized as previously described [3a]. (+)-**7** $[\alpha]_D^{20}$ = + 19 (c 0.4, $CHCl_3$): synthesized as previously described [3a].

Microorganisms, culture and incubation conditions. Screening of the following microorganisms from international collections was effected using a standard liquid medium [18] (100 ml) in orbitally shaken (210 r.p.m.) conical flasks, inoculated with spores and incubated for 64 hours at 27°: *Beauveria bassiana* ATTC 7159, *Mucor plumbeus* CBS 110-16, *Curvularia lunata* NRRL 2380, *Aspergillus candidus* ATCC 20023, *Absidia cylindrospora* MMP 1569, *Aspergillus niger* ATCC 9142, *Actinomucor elegans* MMP 3122, *Rhizopus arrhizus* ATCC 1145, *Thamnostylum piriforme* ATCC 8992, *Cunninghamella echinulata* NRRL 3655, *Cunninghamella elegans* ATCC 36112, and *Cunninghamella baineri* ATCC 9244. The above substrates (28 mg) were dissolved in 1 ml of EtOH and incubation was continued for 144 hours. Only the strains mentioned in *Table 1* and *2* led to biotransformations which were monitored by TLC and GLC; the other microorganisms did not metabolize the starting materials.

Isolation and purification of biotransfrormation products. The incubation medium was filtered and the filtrate, after saturation with NaCl, was extracted with 3 x 100 ml of EtOAc. The combined extracts were dried (K_2CO_3) and evaporated. The crude material was separated into its components by prep. TLC (silica gel; $CHCl_3$ / EtOH / conc. aq. NH_3 20:2:1).

Characterization of the isolated metabolites:

(+)-7-Hydroxymakomakine ((+)-2)

M.p.: 97-98° (EtOAc). $C_{20}H_{26}N_2O$ (310.41)

$[\alpha]_D$: + 106.6 (c = 1.05, $CHCl_3$).

UV (EtOH): 295 (3.44), 276 (3.40), 222 (4.20).

IR ($CHCl_3$): 3590, 3475, 3350 (br.), 2910, 1628, 1589, 1558, 1494, 1450, 1428, 1378, 1309, 1258, 1154, 1143, 950, 904.

^{1}H-NMR (400 MHz): 7.73 (br. *s*, 1 H); 7.43 (*d*, *J*= 8.5, 1 H); 6.83 (*m*, 1 H); 6.72 (*dd*, *J*= 2.2, 0.3, 1 H); 6.65 (*dd*, *J*= 8.5, 2.2, 1 H); 4.78 (*t*, *J*= 2.5, 1 H); 4.58 (*t*, *J*= 2.5, 1 H); 3.45 (*ddd*, *J*= 7.5, 6.3, 2.8, 1 H); 2.98 (*tdm*, *J*= 14.0, 6.5, 1 H); 2.70 (*ddd*, *J*= 14.2, 6.3, 0.7, 1 H); 2.59 (*ddd*, *J*= 14.2, 7.5, 0.5, 1 H); 2.26 (*m*, 1 H); 2.19 (br. *dd*, *J*= 14.4, 5.9, 1 H); 2.12 (*dq*, *J*= 12.7, 3.0, 1 H); 2.07 (*m*, 1 H); 1.59 (*dt*, *J*= 12.7, 3.2, 1 H); 1.50 (*ddd*, *J*= 13.5, 6.1, 4.0, 1 H); 1.40 (*m*, 1H); 1.16 (*s*, 3 H); 1.12 (*s*, 3 H).

NOE: a) irrad. at 7.43 (*H*-C(5)): difference signals at 6.65 (*H*-C(6)), 3.45 (*H*-C(11)), 2.70 (*H*-C(10)), and 2.59 (*H*-C(10')). b) irrad. at 7.73 (*H*-N(1)): difference signals at 6.83 (*H*-C(2)) and 6.72 (*H*-C(8)).

^{13}C-NMR (100 MHz): 152.1 *(s)*, 150.1 *(s)*, 137.3 *(s)*, 122.4 *(s)*, 121.2 *(d)*, 119.8 *(d)*, 113.6 *(s)*, 109.3 *(d)*, 109.1 *(t)*, 97.0 *(d)*, 54.2 *(d)*, 53.2 *(s)*, 43.1 *(d)*, 36.6 *(d)*, 33.1 *(t)*, 32.0 *(t)*, 31.3 *(t)*, 29.7 *(q)*, 29.2 *(t)*, 27.0 *(q)*.

EI–MS: 310 (5, M^+), 295 (3), 175 (7), 174 (7), 164 (100), 147 (19), 146 (40), 93 (14), 91 (13), 41 (19).

(+)-19$_{exo}$-Hydroxymakomakine ((+)-3)

M.p.: 64-65° (Et_2O). $C_{20}H_{26}N_2O$ (310.41)

$[\alpha]_D$: + 33.2 (c= 1.04, $CHCl_3$).

UV (EtOH): 290 (3.46), 281 (3.52), 275 (*sh*, 3.50), 222 (4.30).

IR ($CHCl_3$): 3600, 3475, 2920, 1452, 1429, 1413, 1379, 1355, 1335, 1114, 1087, 1032, 1013, 969, 905, 893.

^{1}H-NMR (500 MHz): 7.96 (br. *s*, 1 H); 7.62 (br. *d*, *J*= 7.8, 1 H); 7.36 (br. *d*, *J*= 8.1, 1 H); 7.19 (*ddd*, *J*= 8.1, 7.1, 1.0, 1H); 7.11 (*ddd*, *J*= 7.9, 7.1, 0.9, 1 H); 7.00 (*d*, *J*= 2.0, 1 H); 4.86 (*t*, *J*= 2.4, 1 H); 4.75 (*t*, *J*= 2.4, 1 H); 4.25 (*m*, 1 H); 3.50-3.47 (*m*, 2 H); 2.77 (*dd*, *J*= 14.3, 5.5, 1 H); 2.61 (*dd*, *J*= 14.3, 8.3, 1 H); 2.28-2.24 (*m*, 2 H); 2.05 (*dt*, *J*= 13.9, 3.2, 1 H); 1.91 (*dm*, *J*= 13.9, 1 H); 1.50 (*m*, 1 H); 1.17 (*s*, 3 H); 1.08 (*s*, 3 H).

^{13}C-NMR (100 MHz): 146.6 *(s)*, 136.4 *(s)*, 127.8 *(s)*, 122.3 *(d)*, 121.9 *(d)*, 119.2 (2*d*), 113.6 *(s)*, 111.8 *(t)*, 111.0 *(d)*, 68.8 *(d)*, 53.9 *(d)*, 51.7 *(s)*, 43.8 *(d)*, 43.3 *(d)*, 40.5 *(t)*, 31.5 *(t)*, 29.2 *(q)*, 27.3 *(q)*, 26.3 *(t)*.

GC–MS: 310 (2, M^+), 295 (2), 180 (100), 162 (8), 159 (16), 130 (63), 117 (10), 85 (10), 77 (12).

(–)-7-Hydroxyhobartine ((–)-5):

M.p.: greenish oil. $C_{20}H_{26}N_2O$ (310.41)

$[\alpha]_D$: – 47 (c = 0.30, $CHCl_3$).

UV (EtOH): 295 (3.44), 276 (3.40), 222 (4.20).

IR ($CHCl_3$): 3540, 3480, 3450, 2910, 1626, 1589, 1495, 1450, 1379, 1310, 1254, 1241, 1157, 1082, 949, 903.

^{1}H-NMR (400 MHz): 7.68 (br. *s*, 1 H); 7.38 (*d*, *J*= 8.4, 1 H); 6.84 (*m*, 1 H); 6.62 (*dd*, *J*= 8.5, 2.1, 1 H); 6.58 (*d*, *J*= 2.1, 1H); 5.64 (*m*, 1 H); 6.35 (*d*, *J*= 9.6, 1 H); 3.49 (*ddd*, *J*= 7.7, 6.7, 2.4, 1 H); 2.75 (*ddd*, *J*= 15.1, 6.5, 0.6, 1 H); 2.65 (*dd*, *J*= 15.1, 7.5, 1 H); 2.28 (br. *d*, *J*= 18.1, 1 H); 2.19 (*m*, 1 H); 2.12-2.07 (*m*, 2 H); 1.80 (*q*, *J*= 1.8, 3 H); 1.62 (*dt*, *J*= 12.5, 3.3, 1 H); 1.46 (*dt*, *J*= 6.2, 3.2, 1 H); 1.20 (*s*, 3 H); 1.12 (*s*, 3 H).

^{13}C-NMR (100 MHz): 152.3 *(s)*, 137.3 *(s)*, 133.5 *(s)*, 124.9 *(d)*, 122.0 *(s)*, 121.0 *(d)*, 119.4 *(d)*, 113.4 *(s)*, 109.5 *(d)*, 97.1 *(d)*, 54.6 *(d)*, 54.0 *(s)*, 38.2 *(d)*, 35.2 *(d)*, 31.6 *(t)*, 29.8 *(q)*, 29.2 *(t)*, 27.9 *(t)*, 25.7 *(q)*, 25.6 *(q)*.

EI–MS: 310 (1, M^+), 295 (1), 175 (4), 164 (100), 160 (22), 159 (18), 148 (12), 147 (35), 146 (81), 133 (13), 93 (12), 91 (11).

(–)-19$_{exo}$-Hydroxyhobartine ((–)-6). Identical with a synthetic reference sample [8].

(+)-10$_{exo}$-Hydroxyaristoteline ((+)-8). Identical with a synthetic reference sample [10].

(+)-14-Hydroxyaristoteline ((+)-9):

M.p.: yellow foam. $C_{20}H_{26}N_2O$ (310.41)

$[\alpha]_D$: + 11.4 (c = 0.63, $CHCl_3$)

UV (EtOH): 289 (3.51), 281 (3.56), 226 (4.28)

IR ($CHCl_3$): 3600, 3490, 3320, 2985, 2925, 1460, 1381, 1350, 1296, 1167, 1104, 940.

^{1}H-NMR (400 MHz): 7.79 (br. *s*, 1 H); 7.44 (*dm*, *J*= 7.6, 1 H); 7.29 (*dm*, *J*= 7.9, 1 H); 7.12 (*ddd*, *J*= 7.9, 7.1, 1.3, 1 H); 7.06 (*ddd*, *J*= 7.6, 7.1, 1.3, 1 H); 3.63 (br. *d*, *J*= 5.9, 1 H); 3.07 (*dd*, *J*= 16.4, 5.7, 1 H); 2.61 (*d*, *J*= 16.4, 1 H); 2.44 (*td*, *J*= 14.1, 5.6, 1 H); 2.05-1.99 (*m*, 3 H); 1.89 (*m*,1 H); 1.77 (br. *dd*, *J*= 13.5, 6.0, 1 H); 1.65 (*td*, *J*= 13.4, 6.1, 1 H); 1.47 (*s*, 3 H); 1.32 (*s*, 3H); 1.06 (*s*, 3H).

^{13}C-NMR (100 MHz): 141.8 *(s)*, 136.1 *(s)*, 128.1 *(s)*, 121.2 *(d)*, 119.2 *(d)*, 118.1 *(d)*, 110.5 *(d)*, 104.5 *(s)*, 70.8 *(s)*, 57.4 *(s)*, 49.9 *(d)*, 44.6 *(d)*, 37.9 *(t)*, 35.6 *(t)*, 34.0 *(t)*, 32.7 *(s)*, 28.1 *(t)*, 25.0 *(q)*, 24.9 *(q)*, 23.0 *(q)*.

FAB–MS: 311 (25, M^++1), 309 (12), 281 (11), 221 (11), 207 (16), 180 (23), 130 (20), 95 (34), 91 (34), 81 (41), 73 (100).

(–)-Tasmanine ((–)-10). Identical with a synthetic reference sample [13].

(+)-11,12-Didehydrotasmanine ((+)-11):

M.p.: 230-231° (Et_2O, colorless crystalline plates). $C_{20}H_{24}N_2O$ (308.41)

$[\alpha]_D$: + 48.2 (c = 1.67, $CHCl_3$)

UV (EtOH): 285 (3.29), 264 (sh, 3.92), 253 (4.06).

IR ($CHCl_3$): 3410, 2930, 2910, 1719 (sh), 1705, 1618, 1469, 1458, 1380, 1330, 1319, 1178, 907.

^{1}H-NMR (400 MHz): 8.36 (br.*s*, 1 H); 7.27(*dm*, *J*= 7.5, 1 H); 7.19 (*ddm*, *J*= 7.7, 1.2, 1 H); 6.97 (*td*, *J*= 7.6, 1.0, 1 H); 6.86 (*dd*, *J*= 7.7, 0.4, 1 H); 3.24 (br. *d*, *J*= 4.8, 1 H); 3.13 (*d*, *J*= 14.6, 1 H); 2.35 (*d*, *J*= 14.6, 1 H); 2.16 (*ddt*, *J*= 13.2, 5.0, 2.7, 1 H); 1.94 (br. *td*, *J*= 13.8, 5.4, 1 H); 1.87 (*m*, 1 H); 1.62 (*ddd*, *J*= 13.2, 3.2, 1.0, 1 H); 1.59 (*m*, 1 H); 1.49 (*tdd*, *J*= 14.1, 5.5, 3.9, 1 H); 1.38 (*s*, 3 H); 1.29 (*s*, 3 H); 0.96 (*s*, 3 H); 0.82 (*ddm*, *J*= 13.3, 5.1, 1 H).

^{13}C-NMR (100 MHz): 183.0 *(s)*, 173.9 *(s)*, 141.6 *(s)*, 130.2 *(s)*, 128.0 *(d)*, 125.7 *(d)*, 121.9 *(d)*, 109.3 *(d)*, 58.7 *(s)*, 56.6 *(s)*, 43.6 *(s)*, 41.2 *(t)*, 40.9 *(d)*, 35.8 *(d)*, 30.3 *(t)*, 29.2 *(q)*, 29.0 *(q)*, 25.7 *(t)*, 21.8 *(t)*, 16.8 *(q)*.

GC–MS: 308 (100, M^+), 293 (13), 253 (14), 196 (21), 176 (13), 173 (13), 172 (62), 145 (32), 137 (60), 136 (28), 117 (33), 81 (97).

In vitro synthesis of (+)-11,12-didehydrotasmanine ((+)-11):

To a soln. of 23 mg (0.074 mmol) of synthetic (–)-tasmanine ((–)-10) [13] in 5 ml of THF were added 51 mg (0.37 mmol) of K_2CO_3 and 100 mg (0.64 mmol) of I_2. The heterogenic mixture was stirred at 25° for exactly 5 min and then quenched by adding 10 ml of an aq. 1 N sodium thiosulfate solution. The mixture was worked up with CH_2Cl_2 and the crude product chromatographed (silica gel, EtOAc) to give 19.6 mg (82.5%) of crystalline (–)-11 which was identical with the sample isolated from the incubation mixture (evidence: TLC, mixed m.p., $[\alpha]_D$, and ^{1}H-NMR spectrum).

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