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Hydroxylation of Yohimbine in Superacidic Media: One-Step Access to Human Metabolites 10 and 11-Hydroxyyohimbine

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Two major human metabolites of yohimbine (**1**), 10- and 11-hydroxyyohimbine (**2** and **3**), were prepared by direct hydroxylation of **1** under superacidic conditions. In this medium, the four positions of the benzene part of yohimbine were hydroxylated and the corresponding monohydroxylated compounds (**2**−**5**) were isolated. The structures of **2**−**5** were elucidated by spectroscopic methods including 1D and 2D NMR techniques.

Yohimbine (**1**) is an indole alkaloid extracted principally from the inner bark of the yohimbe tree, which grows wild throughout Africa. Yohimbine is known as a potent and selective α_2 adrenoreceptor antagonist agent,[1] but it does not show selectivity among the three α_2 adrenoreceptor subtypes.[2] Yohimbine is also well-known for its activity against male impotence and, recently, against female sexual disorders.[3]

Mass spectral analyses of two human metabolites of yohimbine (**2** and **3**), and comparison with authentic samples, indicated that these were 10- and 11-hydroxyyohimbine.[4] Compounds **2** and **3** were studied as selective α_2 adrenoreceptor antagonists,[5,6] and the activity of 11-hydroxyyohimbine (**3**) on these receptors has been patented.[7]

The biological hydroxylation of yohimbine by fungi has been recognized for many years. In this way, Loo and Reidenberg,[8] Patterson et al.,[9] and Hartmann et al.[10] obtained **2** or **3**. Adam et al.[11] reported the NMR spectra of **2**. Concurrently, Levy et al. prepared authentic samples of **2** and **3** by demethylation of methoxy compounds[12] isolated from natural sources.[13] Due to the scarcity of the natural compounds and the poor yields of demethylation reactions, we decided to reinvestigate the partial synthesis of **2** and **3** by applying the unusual reactivity of superacids.

Results and Discussion

The advantages of electrophilic hydroxylation of aromatics with hydrogen peroxide in superacidic media were described by Olah et al.[14,15] We previously described the electrophilic hydroxylation of indole derivatives by hydrogen peroxide under superacidic conditions.[16,17] The oxidizing agent, source of "OH^+", reacted at the benzene ring on the protonated indole principally at positions 5 and 6, corresponding to positions 10 and 11 for yohimbine. It was observed also that the more substituted indoles gave better yields. Using the same strategy with **1** in the presence of hydrogen peroxide, a mixture of hydroxylated derivatives was obtained, together with a large quantity of tar. By using sodium persulfate in place of hydrogen peroxide, yohimbine hydrochloride in place of **1**, and optimization of the conditions, a simple mixture of monohydroxylated yohimbine derivatives was obtained (Scheme 1), the four positions of the benzene ring of yohimbine being hydroxylated. The yield of each compound in the crude mixture after hydrolysis of the superacidic medium was evaluated by analytical HPLC. The mixture contained around 32% 10-hydroxyyohimbine (**2**), 23% 11-hydroxyyohimbine (**3**), 3% 12-hydroxyyohimbine (**4**), and 11% 9-hydroxyyohimbine (**5**). Yohimbine (3%) remained, giving a total of 72% identified compounds. Preparative chromatography, then recrystallization, yielded pure compounds **2** and **3**. Repeated chromatography yielded nearly pure compounds **4** and **5**.

Yohimbine (**1**) and compounds **2**−**5** were studied under identical conditions by various NMR techniques using MeOH as solvent. The stereochemistry of the polycyclic structure was studied by NOESY experimentation. The ^{1}H (Table 1) and ^{13}C (Table 2) NMR chemical shifts were assigned on the basis of 1D (^{1}H, ^{13}C, and DEPT) and 2D (COSY, HMQC, and HMBC) spectra. Elucidation of the position of hydroxylation was carried out using COSY and HMBC spectra. For each aromatic carbon atom, the chemical shift variation between each hydroxyyohimbine derivative and yohimbine confirmed the assignments. Strong NOE between the protons in position 6 and the proton in position 9 for compounds **2**, **3**, and **4** provided further proof permitting differentiation of compounds **4** and **5**. For the nonaromatic part of the yohimbine derivatives, analyses of the differences of ^{13}C and ^{1}H chemical shifts between each hydroxyyohimbine derivative and yohimbine confirmed that this part of yohimbine remained unchanged (Tables 1 and 2). In addition, NOESY experiments showed strong NOE between the axial protons on each face of the nonaromatic polycyclic part of the yohimbine derivatives.

It should be emphasized that the use of superacidic media permitted oxidation of the aromatic ring without modification of the remainder of yohimbine. In fact, protonation of all the functional groups present in the molecule appears to protect fragile centers and to favor electrophilic hydroxylation. The 17-hydroxy group remained intact, without dehydration, and the configuration of the five asymmetric centers was unchanged. This strategy provided direct access to the two identified yohimbine metabolites (**2** and **3**), in one step and with a relatively good yield, along with two compounds (**4** and **5**) that have not been previously reported.

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Scheme 1

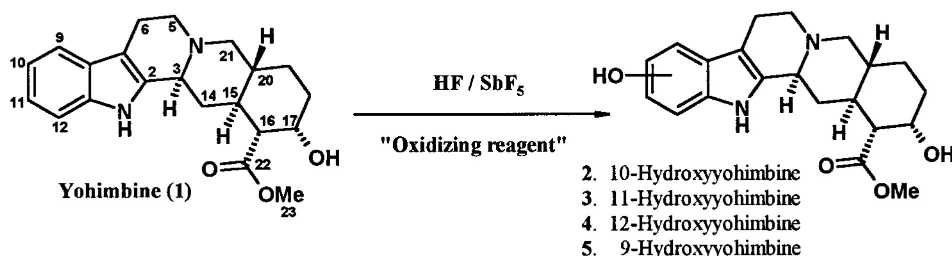

Table 1. ^{1}H NMR Data (δ) of Compounds **1**−**5** in CD_3OD

	1	2	3	4	5
H-3α ax	3.3	3.3	3.4	3.4	3.3
H-5α ax	2.6	2.5	2.7	2.6	2.6
H-5β eq	3.0	3.0	3.1	3.1	3.0
H-6α eq	2.7	2.9	2.7	2.7	3.2
H-6β ax	3.0	2.6	3.0	3.0	3.0
H-9	7.4	6.8	7.2	6.9	
H-10	7.0		6.6	6.8	6.3
H-11	7.0	6.6		6.5	6.8
H-12	7.3	7.1	6.8		6.8
H-14α eq	2.4	2.4	2.5	2.5	2.4
H-14β ax	1.1	1.1	1.2	1.2	1.2
H-15α ax	2.0	1.9	2.0	2.0	2.0
H-16β ax	2.3	2.3	2.4	2.4	2.3
H-17β eq	4.2	4.2	4.3	4.2	4.2
H-18α eq	1.9	1.9	2.0	1.9	1.9
H-18β ax	1.6	1.6	1.7	1.7	1.6
H-19α ax	1.5	1.5	1.6	1.5	1.5
H-19β eq	1.4	1.3	1.4	1.4	1.4
H-20β ax	1.5	1.5	1.6	1.5	1.5
H-21α ax	2.2	2.1	2.3	2.3	2.2
H-21β eq	2.9	2.8	3.0	2.9	2.9
H-23	3.8	3.8	3.8	3.8	3.8

Table 2. ^{13}C NMR Data (δ) of Compounds **1**−**5** in CD_3OD

	1	2	3	4	5
C-2	135.7	136.6	134.1	135.1	133.5
C-3	61.8	61.9	62.0	62.1	62.0
C-5	54.5	54.2	54.3	54.2	54.6
C-6	22.4	22.5	22.5	22.7	24.5
C-7	107.8	107.2	107.6	108.5	107.6
C-8	128.4	129.1	122.5	130.5	118.0
C-9	118.6	103.2	119.0	110.6	152.5
C-10	119.8	151.2	109.7	120.6	104.4
C-11	121.9	111.6	153.8	107.1	122.5
C-12	112.1	112.5	97.9	144.4	104.4
C-13	138.1	133.0	139.2	127.7	140.2
C-14	34.7	34.6	34.8	34.8	34.7
C-15	37.5	37.4	37.5	37.5	37.5
C-16	53.8	53.8	53.9	54.0	53.9
C-17	68.7	68.7	68.7	68.8	68.7
C-18	33.5	33.5	33.5	33.6	33.5
C-19	24.4	24.4	24.5	24.5	24.5
C-20	41.2	41.1	41.3	41.3	41.2
C-21	62.3	62.3	62.4	62.4	62.4
C-22	175.4	175.4	175.4	175.6	175.4
C-23	52.2	52.2	52.2	52.3	52.2

Experimental Section

General Experimental Procedures. ***The authors draw the readers' attention to the dangerous features of superacidic chemistry. Handling of hydrogen fluoride and antimony pentafluoride must be done by experienced chemists with all the necessary safety arrangements in place.***

Anhydrous hydrogen fluoride (Praxair), antimony pentafluoride (Merck ref: 812034), yohimbine (Sigma ref: Y3125), and sodium persulfate (Degussa ref: EWG Nr. 231-892-1) were used as received. ^{1}H and ^{13}C NMR, DEPT, COSY, HMQC, HMBC, and NOESY were measured at 25 °C using a Bruker Avance 400 spectrometer. TMS was used as internal standard, and samples were dissolved in CD_3OD. Analytical HPLC conditions: Merck LaChrom system with a photodiode array detector (L 7455, L7100, L7200, D7000), Waters Symmetry C18 5 μm (250 × 4.6 mm, ref: WAT 054215), gradient (0 min, MeOH 10%, KCl 75 mM pH 2.5 with HCl (KCl) 82%, acetonitrile (AN) 8%; 15 min, MeOH 20%, KCl 72%, AN 8%; 20 min, MeOH 40%, KCl 52% AN 8%; 30 min, MeOH 40%, KCl 52% AN 8%), flow 1 mL/min. Retention time: 10-hydroxyyohimbine (**2**), 9.6 min; 11-hydroxyyohimbine (**3**), 10.9 min; 9-hydroxyyohimbine (**5**), 14.7 min; 12-hydroxyyohimbine (**4**), 16.3 min; and yohimbine (**1**), 23.5 min. Yields in the crude mixture after hydrolysis of the superacidic medium were estimated after isolation and purification of each of the four hydroxylated yohimbine derivatives. UV spectra of compounds **2**, **3**, **4**, **5**, and yohimbine (**1**) differ significantly, and calibration of the chromatogram was accomplished by an external standard method using these five compounds as standards. Preparative HPLC conditions: pump and detector system, Merck SepTech (NovaPrep 5000); pre-packed column, RT Merck Hibar (250 × 25, Lichrospher RP18, 5 μm); column, Prochrom (LC 50-VE); silica gel, Merck Si 60 (15−25 μm ref: 109336); and reversed phase, C18 Lichroprep RP18 (15−25 μm ref: 113901). Mass spectrometry data were obtained on a Finnigan MAT TSQ 7000. For APCI, the temperature of the vaporizer and capillary were respectively 400 and 150 °C. Corona current was set to 5 μA. For DCI, the energy of the electron beam and ion source temperature were respectively set to 70 eV and 150 °C, the pressure of the reagent gas (CH_4) was 2400 mT, and the desorption parameter was 50−500 mA in 0.2 min.

Hydroxylation of 1. Anhydrous hydrogen fluoride (200 mL) was introduced into a Teflon reactor and mechanically stirred at −45 °C. Antimony pentafluoride (260 g) was cautiously added over 3 min, and the temperature reached −10 °C. At −45 °C, sodium persulfate (7 g, 0.029 mol) was added. Then, a solution of yohimbine hydrochloride (10 g, 0.025 mol) in anhydrous hydrogen fluoride (60 mL) was introduced, maintaining the temperature below −35 °C. This mixture was stirred for 1 h and then, very carefully, poured into a vigorously stirred mixture of Na_2CO_3 (500 g), H_2O (500 mL), and ice (2 kg). The aqueous phase was extracted three times with a mixture of ethyl acetate/2-butanone (3:1, 3 × 100 mL). The combined organic phase was washed three times with saline, dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved and diluted to exactly 200 mL with MeOH. The solution was chromatographed to determine the amounts of remaining **1** and of each of the hydroxylated derivatives (**2**−**5**). Merck Si 60 silica gel (75 g) was added to the rest of the solution, and the solvent was evaporated to dryness.

Isolation and Purification. The crude mixture supported on silica gel was introduced onto the top of the chromatographic column (Prochrom LC 50-VE) packed with Merck Si 60 silica gel (250 g) and eluted (50 mL/min) with a gradient from 100% $CHCl_3$ to 90% $CHCl_3$, 8% MeOH, 2% aqueous NH_3 over 2 h. The fractions were selected in three groups after analytical chromatography. The first group of fractions contained principally 12-hydroxyyohimbine (**4**). The second group of fractions contained a mixture of 9-hydroxyyohimbine (**5**) (12%), 10-hydroxyyohimbine (**2**) (33%), and 11-hydroxyyohimbine (**3**) (55%). The third group of fractions contained a mixture of **5** (3%), **2** (67%), and **3** (30%).

12-Hydroxyyohimbine (4). The first group of fractions was evaporated and the residue was subjected to reverse phase chromatography using a prepacked column (250 × 25, Lichrospher RP18, 5 μm) eluted with a gradient from 95% A, 5% B to 90% A, 10% B over 1 h (A: aqueous 1 M CH_3COOH; B: MeOH) to obtain 95% pure **4**. The final purification was done by chromatography with Merck Si 60 silica gel using a gradient from 100% $CHCl_3$ to 90% $CHCl_3$, 8%, MeOH, 2% aqueous NH_3 over 1 h to obtain 97.5% pure **5** (80 mg, 0.77% yield): white amorphous solid; APCI+ *m*/*z* 371.0 [MH^+]; ^1H and ^{13}C NMR, Tables 1 and 2; *anal.* C 67.85%, H 7.18%, N 7.63%, calcd for $C_{21}H_{26}N_2O_4$, C 68.09%, H 7.07%, N 7.56%.

10-Hydroxyyohimbine (2). The third group of fractions was evaporated, and the residue was subjected to crystallization in MeOH to obtain 97% pure **3**. Recrystallization using 2-butanone afforded 98.5% pure **3** (1.4 g, 13.5% yield): colorless crystals; decomposition before melting; APCI+ *m*/*z* 371.3 [MH^+]; ^1H and ^{13}C NMR, Tables 1 and 2; *anal.* C 68.25.05%, H 7.27%, N 7.31%, calcd for $C_{21}H_{26}N_2O_4$, C 68.09%, H 7.07%, N 7.56%.

11-Hydroxyyohimbine (3). The second group of fractions was evaporated, and the residue was subjected to a second chromatography with Merck Si 60 silica gel using a gradient from 100% $CHCl_3$ to 90% $CHCl_3$, 8% MeOH, 2% aqueous NH_3 over 1 h to obtain a mixture of **5** (3%), **2** (22%), and **3** (75%). This mixture, crystallized with MeOH, afforded 95% pure **3**. The final purification was done by chromatography using a prepacked column (250 × 25, Lichrospher RP18, 5 μm) eluted with a gradient from 100% A, 0% B to 85% A, 15% B over 1 h, then recrystallization using MeOH, affording 98% pure **3** (750 mg, 7% yield): colorless crystals; decomposition before melting; APCI+ *m*/*z* 371.0 [MH^+]; ^1H and ^{13}C NMR, Tables 1 and 2; *anal.* C 65.31%, H 7.88%, N 7.03%, calcd for $C_{21}H_{26}N_2O_4$,CH_3OH, C 65.65%, H 7.51%, N 6.96%.

9-Hydroxyyohimbine (5). A selection of chromatographic fractions afforded a mixture of **5** (19%), **2** (44%), and **3** (37%). This mixture was subjected to three reverse phase chromatographies using a prepacked column (250 × 25, Lichrospher RP18, 5 μm) eluted with a gradient from 90% C, 10% B to 75% C, 25% B (C: aqueous KCl 0.5 MeOH pH 2.8 with HCl; B: MeOH) to obtain 95% pure **5**. The final purification was done by chromatography with Merck Si 60 silica gel and a gradient from 100% $CHCl_3$ to 90% $CHCl_3$, 8% MeOH, 2% aqueous NH_3 over 1 h to obtain 97.7% pure **5** (70 mg, 0.67% yield): white amorphous solid; DCI+ *m*/*z* 371.1 [MH^+]; ^1H and ^{13}C NMR, Tables 1 and 2; *anal.* C 65.31%, H 7.88%, N 7.03%, calcd for $C_{21}H_{26}N_2O_4$, C 68.09%, H 7.07%, N 7.56%.

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