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Total Synthesis of the Ortho-Hydroxylated Protoberberines (S)-Govaniadine, (S)-Caseamine, and (S)-Clarkeanidine via a Solvent-Directed Pictet–Spengler Reaction

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Supporting Information

ABSTRACT: The common para regioselectivity in Pictet–Spengler reactions with dopamine derivatives is redirected to the ortho position by a simple change of solvents. In combination with a chiral auxiliary on nitrogen, this ortho-selective Pictet–Spengler produced the 1-benzyltetrahydroisoquinoline alkaloids (S)-crassifoline and (S)-norcrassifoline and the bioactive 1,2-dioxygenated tetrahydropseudoberbine alkaloids (S)-govaniadine, (S)-caseamine, and (S)-clarkeanidine with high enantiopurity. Ortho/para ratios up to 89:19 and diastereomeric ratios up to 85:15 were obtained during formation of the B-ring. The general applicability of this solvent-directed regioselectivity was demonstrated with a second Pictet–Spengler reaction as required for C-ring formation of caseamine (o/p = 14:86 in trifluoroethanol) and clarkeanidine (o/p = 86:14 in toluene).

INTRODUCTION

Most of the 1-benzyltetrahydroisoquinoline alkaloids found in nature are formed from dopamine and contain a 6,7-dioxygenated substitution pattern in the A-ring as a result of enzyme-catalyzed Pictet–Spengler condensations (Figure 1).1,2 Isomeric 1-benzyltetrahydroisoquinolines with oxygen substituents at C-7 and C-8 are less abundant in nature but display interesting biological properties.3 Examples of more complex alkaloids derived from 7,8-dioxygenated 1-benzyltetrahydroisoquinolines are the parent compound crassifoline (3), several tetrahydropseudoberbinere alkaloids (e.g., govaniadine (4)), the caseamine alkaloids (5), and pavine alkaloids such as neocaryachine (6). Labeling studies performed by Müller and Zenk7 to elucidate the biosynthesis of crassifoline and the casearine alkaloids showed that this unusual oxygenation pattern in the tetrahydroisoquinoline ring is not formed by oxygen transposition but most likely by an ortho-selective Pictet–Spenglerase, although this enzyme has not yet been described in literature.

The enantioselective chemical syntheses of 6,7-oxygenated 1-benzyltetrahydroisoquinolines preferably follow the lines of the biosynthesis. In particular, the Bischler–Napieralski method, in combination with asymmetric hydrogenation or by chiral auxiliary directed hydride reduction, is favored for enantioselective preparations (reviewed by Rozwadowska in 2004 and 2016, see ref 2). A practical synthesis of the 7,8-dioxygenated tetrahydroisoquinoline ring system, however, is not accessible via the Bischler–Napieralski reaction, which exclusively yields para products. Likewise, the Pictet–Spengler approach with chiral (organo)catalysis, or with assistance of chiral auxiliaries, is only effective for the traditional 6,7-substitution pattern.2,6,7 A few methods are described to prepare this 7,8-substitution pattern, and these are not based on Pictet–Spengler or Bischler–Napieralski approaches but require multistep quinoline ring construction. Rodrigues described an efficient build-up/chiral auxiliary approach to ortho-hydroxylated crassifoline and the cularine alkaloids.8 Halogen atoms as temporary blocking substituents at positions in the aromatic ring that should stay unsubstituted are also applied.8a

Ortho selectivity toward an activating substituent in Mannich-type cyclizations is more often observed, but in the Pictet–Spengler reaction, ring closure ortho to the phenolic substituent is always a minor process in comparison to the para position. The pH dependency of ortho/para ratios was investigated by Bates, who found pH 7 as an optimum for ortho product formation (o/p = 50:50) using formaldehyde or acetaldehyde.9

In a previous publication on the synthesis of javaberine alkaloids, we reported that the regioselectivity of the Pictet–Spengler reaction between secondary phenylethylamines and aldehydes depends strongly on the solvent and varies between 99% para selectivity in trifluoroethanol to 81% ortho selectivity in aprotic, apolar solvents without addition of external acids (Scheme 1).2a

Furthermore, both ortho and para products were formed as single diastereomers. To translate this uncatalyzed Pictet–Spengler reaction into a useful tool for natural product synthesis, ortho-selective Pictet–Spengler reactions were recently reported in literature. However, with the exception of the benzylated precursors, the products were always para substituted and required additional steps to access ortho products.

Upon screening a variety of aprotic solvents and solvent mixtures for ortho-selectivity toward precursor (1), we found that the ortho-selectivity toward an activating substituent in the aromatic ring that should stay unsubstituted can be directed by a change in solvent (Table 1).

The ortho/para ratio and the diastereomeric ratio were optimized for each precursor, and all reactions were performed at reflux temperature. Promising results were observed with the precursors of dopamine derivatives 1 and 2. In order to remove the benzyl protecting group, the ortho-substituted precursors (S)-1a and (S)-2a were subjected to the Pictet–Spengler reaction conditions. A diastereomeric ratio of 85:15 was obtained for (S)-1a and 89:19 for (S)-2a, respectively. The ortho/para ratio strongly depended on the solvent. The ortho product was the major product in acetic acid, whereas the para product was the major product in acetic anhydride. These results were independent of the nature of the phenolic substituent at C-3 or C-8 of the precursor. Additionally, all ortho-selective precursors react under a wide range of conditions, and the ortho-para ratio is not affected by the change of the phenolic substituent.

In combination with a chiral auxiliary on nitrogen, this ortho-selective Pictet–Spengler reaction produces the 1-benzyltetrahydroisoquinoline alkaloids (S)-crassifoline and (S)-norcrassifoline and the bioactive 1,2-dioxygenated tetrahydropseudoberbine alkaloids (S)-govaniadine, (S)-caseamine, and (S)-clarkeanidine with high enantiopurity. The ortho/para ratios up to 89:19 and diastereomeric ratios up to 85:15 were obtained during formation of the B-ring. The general applicability of this solvent-directed regioselectivity was demonstrated with a second Pictet–Spengler reaction as required for C-ring formation of caseamine (o/p = 14:86 in trifluoroethanol) and clarkeanidine (o/p = 86:14 in toluene).
Spengler procedure\textsuperscript{9} to both the challenging ortho regioselectivity and enantioselectivity in the 1-benzyltetrahydroisoquinoline series, we herein disclose a chiral auxiliary approach starting from a (S)-(-)-\(\alpha\)-methylbenzyl-functionalized dopamine analogue.\textsuperscript{10}

**RESULTS AND DISCUSSION**

The benzene ring in the dopamine part of the key precursor 10 (Scheme 2) requires activation by a free phenolic OH to allow non-acid-catalyzed Pictet–Spengler reactions with dopamine derivatives. If methoxy or methylenedioxy substituents are the activating substituents, strongly acidic catalysts are required that produce almost exclusively para-substituted Mannich-type products.\textsuperscript{2} The required phenylethylamine 10 was prepared from phenylacetaldehyde 9 that was obtained after a convenient Wittig/hydrolysis homologation process\textsuperscript{7,15} starting from isovanilline (7). Reductive amination of phenylacetaldehyde 9 with (S)-\(\alpha\)-methylbenzylamine gave chiral dopamine analogue 10. To optimize the Pictet–Spengler conditions, we selected (S)-govaniadine 4, a 1,2-oxygenated tetrahydroprotoberberine alkaloid that has not been synthesized before (Scheme 2). Govaniadine is isolated from *Corydalis govaniana Wall.* and has been the subject of different studies on its biological activity since its discovery in 2013.\textsuperscript{11} These studies revealed significant analgesic activity for govanadiine, similar to that of ibuprofen, due to its potential binding to the COX-2 enzyme.\textsuperscript{12} Furthermore, high and selective leishmanicidal activity,\textsuperscript{13} antiurease activity,\textsuperscript{10} and glucoronidase inhibition were reported.\textsuperscript{14}

The Pictet–Spengler reaction of aldehyde 11\textsuperscript{16} with equimolar amounts of 10 in different solvents was monitored by NMR and shows a clear solvent-dependent ortho/para distribution of the product (Table 1). Prootic solvents, with TFE as the strongest proton donor, gave fast reactions with high preference for the para isomer 17, which is typical for a process that is acid catalyzed. Reactions in toluene and dichloroethane, both performed at higher dilution to prevent intermolecular catalysis by the phenolic OH, were considerably slower but gave good selectivity for the ortho isomer 15.

Importantly, the diastereomeric ratio of the ortho isomers 15, with the required (S)-configuration at C-1\textsuperscript{17} and 16 (R-configuration at C-1, not shown) in toluene and dichloro-
methanol), indicating that the product isolated from which was reductively methylated to crassifoline α formed as a ca. 50:50 mixture of inseparable diastereomers. Dichloroethane.

α one reported in the literature ([Scheme 4]). Comparable yields and selectivities were obtained from the top side of the iminium ion. In toluene, the highest ortho/para ratio was obtained, while DCE gave a better diastereomeric ratio. Since the yields in both solvents were comparable, toluene was selected for scaling up the synthesis, providing pure 15 in 48% isolated yield. Debenzylation of 15 to 18 and cyclization of the C-ring with formaldehyde under acidic conditions produced (S)-(−)-govaniana 4, which was identical to the natural product (Scheme 3).

Table 1. Ortho/Para Ratios in the Pictet–Spengler Cyclization of 10

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>T (°C)</th>
<th>time</th>
<th>ortho/para</th>
<th>de ortho (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TFE</td>
<td>75</td>
<td>1 h</td>
<td>10:90</td>
<td>53:47</td>
</tr>
<tr>
<td>2</td>
<td>methanol</td>
<td>65</td>
<td>2 d</td>
<td>38:62</td>
<td>60:40</td>
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<tr>
<td>3</td>
<td>MeCN</td>
<td>80</td>
<td>2 d</td>
<td>65:35</td>
<td>60:40</td>
</tr>
<tr>
<td>4</td>
<td>DCE(^b)</td>
<td>80</td>
<td>4 d</td>
<td>72:28</td>
<td>85:15</td>
</tr>
<tr>
<td>5</td>
<td>toluene</td>
<td>105</td>
<td>4 d</td>
<td>81:19</td>
<td>73:27</td>
</tr>
</tbody>
</table>

\(^a\)At >80% conversion, determined by \(^1\)H NMR. \(^b\)Performed at 40 mM. TFE = 2,2,2-trifluoroethanol, DCE = 1,2-dichloroethane.

ethane was good, and the isomers were readily separable by chromatography. This is in sharp contrast with the para isomer 17, which was formed exclusively as an inseparable mixture of both diastereomers in nearly equal amounts. NMR spectra of the crude reaction mixtures at an early stage showed that the reactants were converted into the unstable, the para isomer is formed exclusively when alkoxy groups are used as the activating substituents, as we also have shown in the govaniana synthesis (Scheme 2). When a free phenolic OH is the activator, the para product is always formed in excess, but is accompanied by some ortho product. Application of the solvent-directed Pictet–Spengler process (see Scheme 2) to the tetrahydroprotoberberine synthesis with norcrassifoline and formaldehyde selectively produced both isomers under mild conditions (Scheme 4). The para isomer (S)-caseamine 24 was obtained by reaction of 23 with formaldehyde in trifluoroethanol [64%, o/p = 14:86, >99% ee after recrystallization, \([\alpha]_D^{20} = -314\) \(\text{lit.}^{18} [\alpha]_D^{20} = -328\)]. Starting from 23 under aprotic conditions using paraformaldehyde in toluene, the ortho isomer (S)-clarekanide 25 was formed [55%, o/p = 86:14, 95% ee after crystallization, \([\alpha]_D^{20} = -442\) \(\text{lit.}^{18} [\alpha]_D^{20} = -277\)].

In conclusion, we have shown that Pictet–Spengler reactions under apolar conditions can produce the otherwise difficult to access ortho-oxygenated products. The chiral auxiliary-supported route is straightforward, scalable, and in particular, suitable for high diastereoselectivity in ortho-hydroxylated tetrahydroisoquinoline preparations. In addition, application of this solvent-directed Pictet–Spengler approach to regioselective tetrahydroprotoberberine synthesis provides a useful addition to existing methods.

### EXPERIMENTAL SECTION

#### General Information

Anhydrous CHCl\(_3\) and CH\(_2\)CN were freshly distilled from CaH\(_2\). Dried THF was obtained by distillation from sodium/benzophenone. DMF and DMSO on 4 Å molecular sieves were obtained from Sigma-Aldrich and stored under \(N_2\) atmosphere. Toluene was distilled and stored on 4 Å molecular sieves. Reagents were purchased with the highest purity (usually >98%) from Sigma-Aldrich and Fluorochem and used as received. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254). SilPhas P60 (particle size 40–65 μm) was used for silica column chromatography. NMR spectra were recorded on Bruker DRX-500, -400, and -300 MHz instruments and calibrated on residual undetered solvent signals as internal standard. The \(^2\)H NMR multiplicities were abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet. High-resolution mass spectra (HRMS) were recorded on an AccuTOF GC v 4g JMS-T100GC mass spectrometer (JEOL, Japan). An FD/FI probe equipped with a FD emitter of 10 μm. Current rate 5.12 mA/min over 1.2 min machine using field desorption (FD) as ionization method. IR spectra were recorded on a Bruker Alpha FTIR machine. Chiral HPLC was performed with a Shimadzu LC-20AD with Shimadzu SPD-M20A diode array detector using a Daicel Chiralcel AD column (eluent n-heptane/2-propanol 70/30, flow 1.000 mL/min, λ 230 nm).
2-Methoxy-5-(2-methoxyethenyl)phenol (8). KOt-Bu (22.4 g, 200 mmol) was added in three portions, with intervals of 3 min, to an efficiently stirred suspension of methoxymethyltriphenyl phosphonium chloride (34.3 g, 100 mmol) in dry THF (250 mL) with ice cooling. After additional stirring for 5 min, isovanillin (13.7 g, 90 mmol) was added in three portions, with intervals of 2 min, to the reaction mixture resulting in a rapid color change from red to yellow. The cooling bath was removed, and the mixture was stirred at rt for 5 h. Silica gel was added (150 g), the solvents were evaporated thoroughly, and the residue was put on top of a silica column. Flash chromatography (petroleum ether/ethyl acetate 4/1, 3/1 and 2.5/1) gave 8 (13.3 g, 73.9 mmol, 82%, 45:55 E/Z mixture) as an oil, which solidified upon standing. The spectra were identical with those of ref 15: 1H NMR (400 MHz, CDCl3) δ 7.09 (dd, J = 8.4, 2.1 Hz, 1H), 7.05–6.93 (m, 2H), 6.87–6.69 (m, 3H), 6.12 (s, 1H), 6.08 (d, J = 7.0 Hz, 1H), 5.82 (d, J = 12.9 Hz, 1H), 5.20 (d, J = 7.0 Hz, 1H), 3.82 (s, 3H), 3.82 (s, 3H), 3.74 (s, 3H), 3.68 (s, 3H).

2-(3-Hydroxy-4-methoxyphenyl)acetaldehyde (9). A mixture of TFA (5 mL) and water (5 mL) was added to a solution of enol ether 8 (7.39 g, 41.0 mmol) in DCM (200 mL). The resulting heterogeneous mixture was stirred vigorously overnight at rt. Water was added, and after separation the organic layer was washed with NaHCO3 aq and dried over Na2SO4. Chromatographic separation (2/1 and 3/2 petroleum ether/ethyl acetate) gave pure 9 (3.75 g, 24.7 mmol, 60%) as an oil, which solidified in the freezer: 1H NMR (400 MHz, CDCl3) δ 9.66 (t, J = 2.4 Hz, 1H), 6.83 (dd, J = 8.2, 1.0 Hz, 1H), 6.78 (d, J = 2.1 Hz, 1H), 6.67 (dd, J = 8.2, 2.1 Hz, 1H), 6.12 (s, 1H), 3.83 (s, 3H), 3.55 (d, J = 2.4 Hz, 2H); 13C{1H} NMR (101 MHz, CDCl3) δ 199.7, 145.9, 145.8, 124.6, 120.9, 115.7, 111.0, 55.7, 49.5.

(S)-2-Methoxy-5-(2-((1-phenylethyl)amino)ethyl)phenol (10). Aldehyde 9 (3.74 g, 22.5 mmol) and (S)-(−)-α-methylbenzylamine (3.1 mL, 24 mmol) were dissolved in THF (75 mL) and stirred at 0 °C for 30 min. Sodium triacetoxyborohydride (10.6 g, 50 mmol) was added, and the mixture was stirred at 0 °C for 30 min and at rt for 14 h. The solvent was evaporated, and the residue was dissolved in ethyl acetate and washed with Na2CO3 solution and water. Next, the product was extracted from the organic layer with aqueous HCl (3 × 100 mL). The water layer was washed three times with ethyl acetate before the water layer was basified with Na2CO3 solution. Extraction with ethyl acetate, drying with Na2SO4, and evaporation of the solvent gave chiral amine 10 (5.02 g, 18.5 mmol, 82%) as a solid: mp 86−92 °C; 1H NMR (400 MHz, CDCl3) δ 7.45–7.14 (m, 5H), 6.81–6.73 (m, 2H), 6.66 (d, J = 8.2, 2.3 Hz, 1H), 5.65 (bs, 1H), 3.88 (s, 3H), 3.78 (q, J = 6.7 Hz, 1H), 2.87–2.47 (m, 4H), 1.35 (d, J = 6.7 Hz, 3H); 13C{1H} NMR (75 MHz, CDCl3) δ 146.0, 145.6, 145.0, 132.8, 128.5, 127.0, 126.7, 119.7, 115.5, 111.5, 58.2, 55.9, 48.7, 35.3, 23.9; HRMS (ESI+) m/z calcd for C17H22NO2 (M + H)+ 272.1651, found 272.1642.
A solution of 10 (0.542 g, 2.0 mmol) and homopiperonal 11 (0.345 g, 2.1 mmol) in anhydrous toluene (50 mL) was stirred at 105 °C for 4 days. Evaporation of the solvent and separation by flash chromatography (petroleum ether/ethyl acetate 19:1, 10:1, and 4:1) provided the minor (R)-isomer 16 (0.150 g, 0.962 mmol, 48%), and finally, an inseparable mixture of the two isomers 17 (0.135 g, 0.324 mmol, 16%). 16: [α]D<sup>20</sup> = −2.0 (MeOH, c = 2.1); 1H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.31–7.24 (m, 2H), 7.25–7.19 (m, 3H), 6.97 (s, 1H), 6.87 (d, J = 7.9 Hz, 1H), 6.82 (d, J = 7.9 Hz, 1H), 6.77 (d, J = 8.2 Hz, 1H), 6.64 (d, J = 8.2 Hz, 1H), 6.11–5.92 (m, 2H), 5.80 (s, 1H), 4.50 (dd, J = 10.3, 3.0 Hz, 1H), 3.92 (s, 3H), 3.70 (q, J = 6.4 Hz, 2H), 3.31–3.15 (m, 1H), 3.06 (dd, J = 13.7, 3.0 Hz, 1H), 2.89–2.78 (m, 2H), 2.77–2.70 (m, 2H), 2.27–2.20 (m, 1H), 1.03 (d, J = 6.4 Hz, 3H); 13C{1H} NMR (101 MHz, CDCl<sub>3</sub>) δ 146.9, 146.4, 145.4, 143.9, 142.3, 135.7, 128.4, 128.1, 127.2, 126.5, 125.0, 122.5, 119.4, 119.3, 119.4, 110.8, 107.4, 100.5, 57.7, 56.0, 54.2, 39.6, 39.5, 22.5, 21.8; IR (neat) ν 3514, 1487 cm<sup>−1</sup>; HRMS (FD<sup>+</sup>): m/z calculated for C<sub>26</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub> (M + H)<sup>+</sup> 418.2015, found 418.2014.

A mixture of amine 10 (1.084 g, 4.0 mmol) and aldehyde 19 (0.12 g, 4.0 mmol) was heated at 105 °C in anhydrous toluene (100 mL, 40 mM) during 5 days. Separation by flash chromatography (petroleum ether/ethyl acetate, 12:1, 10/1) provided first the minor (R)-isomer 21 (0.462 g, 0.867 mmol, 21.7%) then the desired (S)-isomer 20 (0.962 g, 0.45 mmol, 45%), and finally an inseparable mixture of two para isomers in a ca. 1:1 ratio (0.221 g, 0.42 mmol,
A mixture of 22 (hydrochloride, 35.1 mg, 0.10 mmol), paraformaldehyde (35 mg, 0.80 mmol), sodium acetate (35 mg, 0.40 mmol), sodium cyanoborohydride (33.0 mg, 0.54 mmol), and zinc chloride (35.0 mg, 0.26 mmol) was stirred in methanol (4 mL) for 24 h at rt. Silica gel was added, and the residue obtained after evaporation was applied to a silica column. Elution with ethyl acetate, ethyl acetate/MeOH/Et$_3$NH$_2$ 95/3/2, and ethyl acetate/MeOH/ Et$_3$NH$_2$ 90/7/3 gave crassifoline (3) (23.7 mg, 0.072 mmol, 72%) as a glass; $[\alpha]_D^{20}$ +17.6 (c = 0.5 in MeOH) [lit. $[\alpha]_D^{20}$ +20 (c = 0.5 in MeOH)]; $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 6.96 (d, $J$ = 1.8 Hz, 1H), 6.78 (d, $J$ = 1.7 Hz, 1H), 6.75 (d, $J$ = 8.5 Hz, 1H), 6.63 (d, $J$ = 8.3 Hz, 1H), 5.79 (bs, 2H), 4.10 (dd, $J$ = 9.4, 3.0 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.30 (ddd, $J$ = 12.9, 10.5, 5.0 Hz, 1H), 3.01 (dd, $J$ = 14.3, 3.0 Hz, 1H), 2.95–2.70 (m, 3H), 2.51–2.41 (m, 1H), 2.37 (s, 3H); $^{13}$C($^1$H) NMR (101 MHz, CDCl$_3$) $\delta$ 145.2, 144.9, 144.2, 142.5, 134.3, 127.2, 124.3, 120.5, 119.2, 115.6, 110.4, 109.0, 60.2, 56.1, 55.9, 44.9, 42.8, 38.9; HRMS (FD$^+$) $m/z$ calc'd for C$_{16}$H$_{24}$NO$_4$ (M + H)$^+$ 370.1800, found 330.1689.

(S)-(−)-Casearidine (24). A solution of 23 (free base, 45 mg, 0.125 mmol) and 37% aqueous formaldehyde (30 μL, 0.40 mmol) in trифлоороэтанол (1.0 mL) was stirred during 5 h at rt. Casearidine 24 (21.8 mg, 0.066 mmol, 53%) directly crystallized from the reaction mixture. Chromatography (ethyl acetate and ethyl acetate/MeOH 97/3 gave additional casearine (4.5 mg, total yield 0.080 mmol, 64%) and clarkeainde 25 (4.4 mg, 0.013 mmol, 10%, spectra see next experiment). Casearidine 24 ee 99% (Charlcel AD column, eluent n-heptane/2-propanol 70:30, flow 1.000 mL/min): $[\alpha]_D^{20}$ −314 (CHCl$_3$ + MeOH, c = 0.15) [lit. $[\alpha]_D^{20}$ −328 (c = 0.04, CHCl$_3$)]; mp 246−250 °C (lit. $^{18}$ mp 246−247 °C); $^1$H NMR (300 MHz, d$_6$-DMSO, partial overlap by solvent peaks) $\delta$ 8.64 (s, 1H), 8.54 (s, 1H), 6.79 (d, $J$ = 8.2 Hz, 1H), 6.62 (s, 1H), 6.55 (d, $J$ = 8.2 Hz, 1H), 6.46 (s, 1H), 3.80 (s, 2H), 3.77 (s, 3H), 3.73 (s, 3H), 3.46–3.36 (m, 1H), 2.96 (dt, $J$ = 10.4, 4.8 Hz, 1H), 2.83 (dt, $J$ = 13.2, 5.6 Hz, 1H), 2.67 (dt, $J$ = 15.8, 4.7 Hz, 1H), 2.40 (dd, $J$ = 16.1, 11.3 Hz, 1H); $^{13}$C($^1$H) NMR (75 MHz, d$_6$-DMSO) $\delta$ 145.8, 145.2, 144.6, 142.8, 127.8, 127.0, 125.6, 124.7, 118.7, 115.2, 110.0, 109.8, 57.0, 56.1, 55.9, 46.9, 42.8, 39.9, 31.4, 29.3; HRMS (FD$^+$) $m/z$ calc'd for C$_{19}$H$_{24}$NO$_4$ (M$^+$) 327.1471, found 327.1499.

(S)-(−)-Clarkeainde (25). A solution of norcrassifoline (23) (free base, 63 mg, 0.20 mmol) in anhydrous toluene (4 mL) was stirred with paraformaldehyde (9.0
mg, 0.30 mmol) at 105 °C for 3 h. The solvent was evaporated, and the isomers were separated by chromatography: petroleum ether/ethyl acetate 1:1 and ethyl acetate for the ortho isomer clarkeanidine 25 (36.1 mg, 0.11 mmol, 55%) and then ethyl acetate/MeOH 97/3 for the para isomer caseamine 24 (6.0 mg, 0.018 mmol, 9%). Clarkeanidine (25); mp 177–180 °C (recrystallized from DCM/petroleum ether); [α]D20 = 178–179 °C); ee 95% (Chiralcel AD column, eluent n-heptane/2-propanol 70:30, flow 1.00 mL/min); [α]D19 = 442 (c = 0.1, CHCl3) [lit.17,18 [α]D20 = 277 (CHCl3)]; 1H NMR (300 MHz, CDCl3) δ 6.75 (m, 2H), 6.66 (m, 2H), 5.78 (bs, 2H), 4.24 (d, J = 16.0 Hz, 1H), 3.99 (dd, J = 11.2, 3.5 Hz, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.32 (m, 1H), 3.12 (m, 1H), 3.12–3.10 (m, 1H), 2.89–2.64 (m, 4H). 13C{1H} NMR (75 MHz, CDCl3) δ 144.3, 143.8, 142.5, 141.7, 129.0, 128.5, 124.6, 121.0, 119.4, 108.9, 56.2, 56.2, 56.1, 53.0, 49.1, 32.2, 29.8; HRMS (FD+) m/z calcd for C19H22NO4 (M + H)+ 328.1549, found 328.1558.

**References**


(17) The 1-(S)-configuration of 15 was determined by conversion to (S)-govaniadine.


