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THE ISOLATION AND CHARACTERIZATION OF A NEW TETRAHYDROPROTOBERBERINE ALKALOID FROM *CORYDALIS CLARKEI*

M.A. ROTHERA, S. WEHRLI, and J.M. COOK*

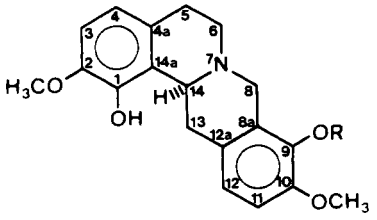
Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201

ABSTRACT.—The MeOH extract of *Corydalis clarkei* Prain yielded two tetrahydroprotoberberine alkaloids that bear the unusual 1,2- and 9,10-tetraoxygenated substitution pattern observed previously only in caseanidine (10). The alkaloids have been identified as caseanidine (**1**) and the new alkaloid, clarkeanidine (**2**). The ^{1}H- and ^{13}C-nmr shifts of both alkaloids have been assigned unambiguously using 2-D nmr. The elucidation of the structure of **2** was achieved by comparison of the ^{1}H- and ^{13}C-nmr spectra of **2** with those of caseanidine coupled with additional spectroscopic evidence.

Corydalis clarkei Prain is the first of a number of Tibetan medicinal plants (1,2) collected in the summer of 1984 (3) to be investigated chemically. In conjunction with other plants, it is purportedly employed to treat "high blood pressure" (4), although as yet, no evidence for its effectiveness has been obtained. It is one of approximately 25 *Corydalis* species that grow in the Himalayan region at an altitude of 12,000-15,000 feet (5,6).

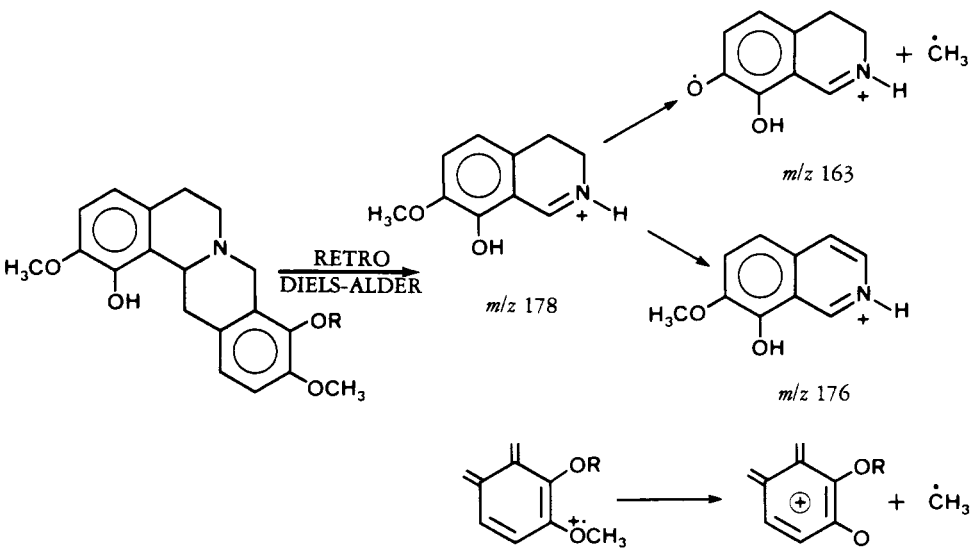
The *Corydalis* genus is a member of the family Fumariaceae, and many alkaloids from this genus are known (7,8). Owing to the association of *C. clarkei* Prain with Tibetan medicine and the plant's unusual geographical location, it was decided to analyze the plant in terms of its alkaloidal content.

The methanolic extract of *C. clarkei* Prain was treated, according to the procedure of Tani *et al.* (9), and yielded two alkaloids: caseanidine (**1**) and a new alkaloid for which we propose the name clarkeanidine (**2**). Alkaloid **1** isolated from the "non-phenolic"



ether extract, appeared to be a compound similar, if not identical, to caseanidine (10): $C_{20}H_{23}NO_4$; ms, *m/z* 341(100), 326(7), 310(20), 178(8), 176(6), 164(82), 149(38). A fragmentation pattern indicative of tetrahydroprotoberberines (11,12) could be easily rationalized (Scheme 1) from these data. The ir spectrum contained an absorbance at 3530 cm^{-1} (OH stretch) and two signals at 2838 cm^{-1} and 2758 cm^{-1} (both weak) which correspond to Bohlmann bands (13). The ^{1}H-nmr spectrum also confirmed the tetrahydroprotoberberine nature of the struture. In order to assign unambiguously the ^{1}H- and ^{13}C-nmr spectra and determine the exact position of the substituents on rings A and D, a detailed ^{1}H- and ^{13}C-nmr study was undertaken. This included homo and hetero 2-D correlated experiments. Comparison of the spectra of an authentic sample of caseanidine (10), kindly provided by Dr. D.B. MacLean, confirmed the assignment. The structure of clarkeanidine was elucidated by comparison of its spectra with those of **1** combined with further spectroscopic evidence. To these authors' knowledge, this paper reports the first definitive 2-D, ^{13}C-nmr, and ^{1}H-nmr study on **1** and **2**.

The ^{1}H-nmr spectrum of **1** (Table 1) possesses many of the features that are useful in distinguishing between tetrahydroprotoberberines. In particular, the aromatic rings



SCHEME 1. Mass spectral fragmentation schemes for caseanidine (**1**) and clarkeanidine (**2**).

contain protons that can only be *ortho* coupled (J=8 Hz). This restricts the number of possible substitution patterns on rings A and D considerably. In the alicyclic region, the significant chemical shift difference between H-8_{ax} and H-8_{eq} (0.42 ppm) is indicative of the influence of a 9-oxygenated substituent (13) on the chemical shifts. The three protons H-13_{ax}, H-13_{eq}, and H-14 form a clear AMX system, and the four methylene protons at C-5 and C-6 give rise to two complex multiplets at δ 2.70 and δ 3.10.

A COSY experiment on **1** (Figure 1) performed with sufficient resolution reveals numerous long range couplings useful in making the assignments. In particular, the aromatic proton at δ 6.63, which appears furthest upfield, is not coupled to any of the methoxyl groups but does show small cross peaks which originate from coupling to a proton in the multiplet at δ 3.10. This is due to coupling of a benzylic proton at C-5

TABLE 1. ^{1}H-nmr Data of Caseanidine (**1**) and Clarkeanidine (**2**)[a]

H_3	6.72 (J=8 AB_q)	6.71 (J=8 AB_q)
H_4	6.63 (J=8 AB_q)	6.62 (J=8 AB_q)
H_{5ax}	2.70 (m)[b]	2.72 (m)[b]
H_{5eq}	3.10 (m)[b]	3.10 (m)[b]
H_{6ax}	2.70 (m)[c]	2.72 (m)[c]
H_{6eq}	3.10 (m)[c]	3.10 (m)[c]
H_{8ax}	3.79 (dJ=16)	3.81 (dJ=16)
H_{8eq}	4.22 (dJ=16)	4.21 (dJ=16)
H_{11}	6.75 (J=8 AB_q)	6.65 (J=8 AB_q)
H_{12}	6.81 (J=8 AB_q)	6.72 (J=8 AB_q)
H_{13ax}	2.70 (dd$J$=16J=11.5)	2.72 (dd$J$=16J=11.5)
H_{13eq}	3.72 (dd$J$=16J=4)	3.70 (dd$J$=16J=4)
H_{14}	3.90 (dd$J$=11.5J=4)	3.96 (dd$J$=11J=4)
2, 10-OCH_3	3.83 (s6H)	3.85 (s3H) 3.86 (s3H)
9-OCH_3	3.85 (s3H)	—
1-OH	5.23 (s)	5.68 (s)
9-OH	—	5.85 (s)

[a]Chemical shifts are in ppm relative to internal $CHCl_3$ (0.2%).
[b,c]Assignments may be interchanged in columns.

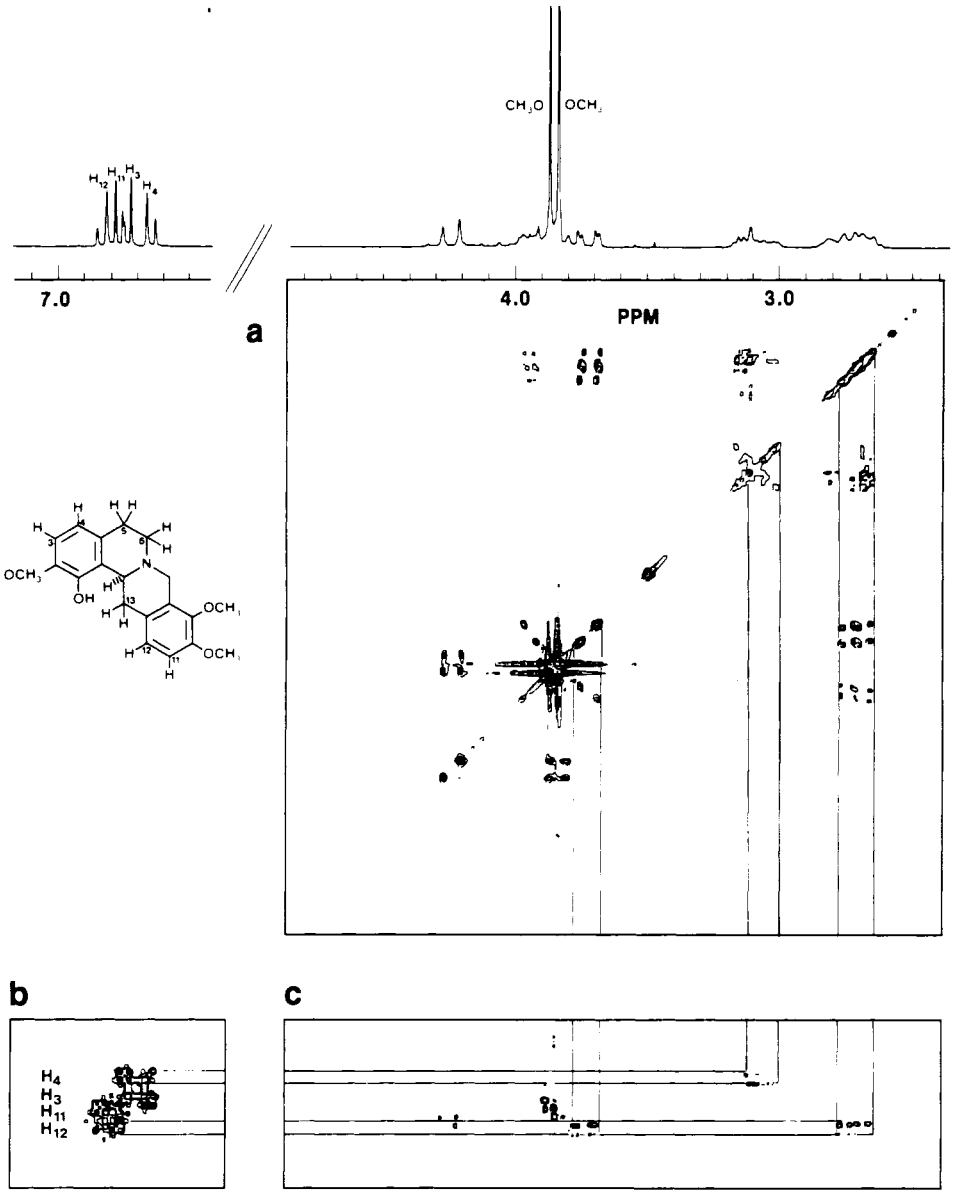


FIGURE 1. COSY spectrum of caseanidine in $CDCl_3$ at 250 MHz with transmitter placed in the center of the spectrum. **a**. Expansion of the alicyclic region, **b**. Expansion of the aromatic region, **c**. Cross peaks.

with the *ortho*-coupled aromatic proton at C-4. The aromatic signal which is furthest downfield also is not coupled to the methoxyl groups but is coupled to the (C-13) proton located at δ 3.72. This defines the signals for H-4 and H-12, and therefore H-3 and H-11; the latter protons are clearly coupled to methoxyl protons. The substitution pattern is therefore 1,2 and 9,10. The large chemical shift difference between H-13_{ax} and H-13_{eq} (1.02 ppm) is noteworthy.

The ^{13}C assignments for **1** are given in Figure 2 and result from 2-D ^{1}H-^{13}C correlated experiments. In these, the magnetization transfer was made using the one-bond coupling constant for the assignment of the proton bearing carbon atoms (Figure 3), and the three-bond coupling constant was employed for the assignment of quaternary

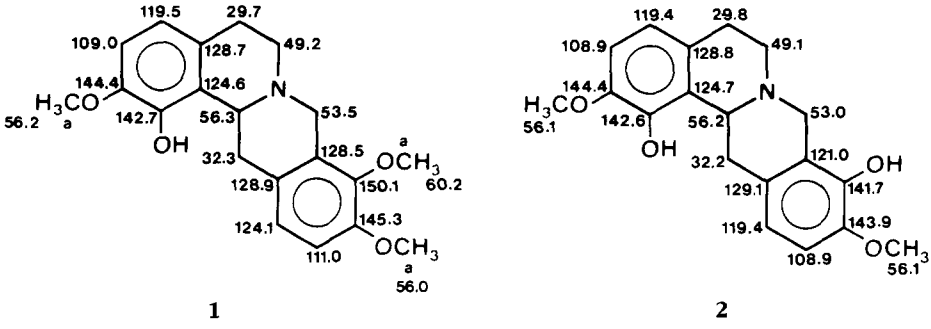


FIGURE 2. ^{13}C-nmr chemicals shifts of caseanidine (**1**) and clarkeanidine (**2**) in ppm.

[a]These two signals can be interchanged.

carbons (Figure 4). In both cases, the carbon assignments are based on the proton assignments made previously.

From the first experiment (Figure 3), it is clear that the signal at 56.3 ppm is due to the overlap of the signals from two methoxyl groups with that of C-14. It was not possible to assign unambiguously the different methoxyl signals because of the poor resolution in the proton dimension. It is interesting to note that protons H-5_{ax} and H-5_{eq} appear at substantially different chemical shifts located at δ 3.10 and δ 2.70; H-6_{ax} and H-6_{eq} are similarly separated. These multiplets have often been quoted incorrectly as representing the two separate methylene groups. In the above experiments, it was not

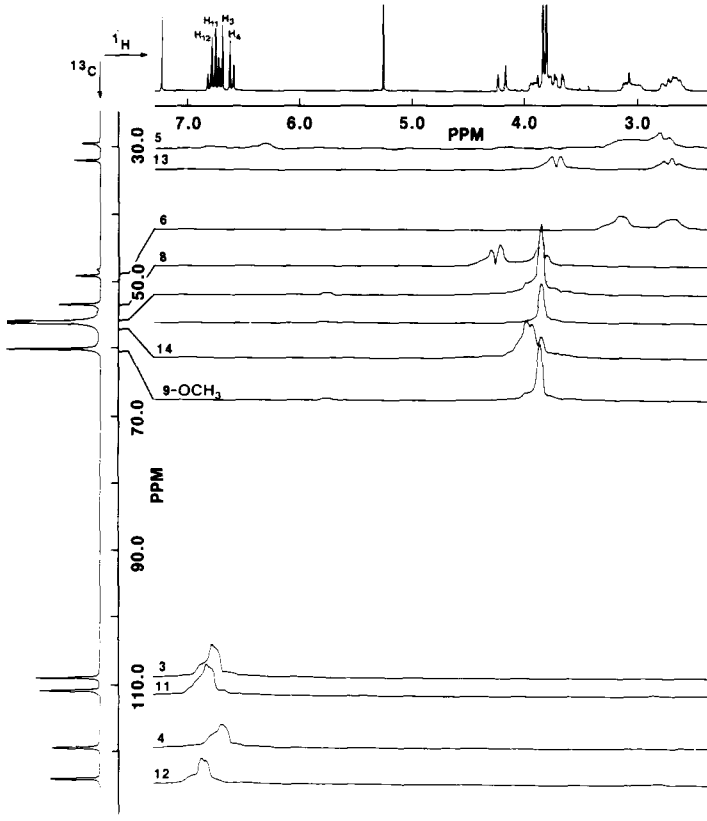


FIGURE 3. 2-D ^{1}H-^{13}C correlated experiment on caseanidine (**1**) using the one-bond coupling constant. Carbon (^{13}C) assignments are given to the left of each trace.

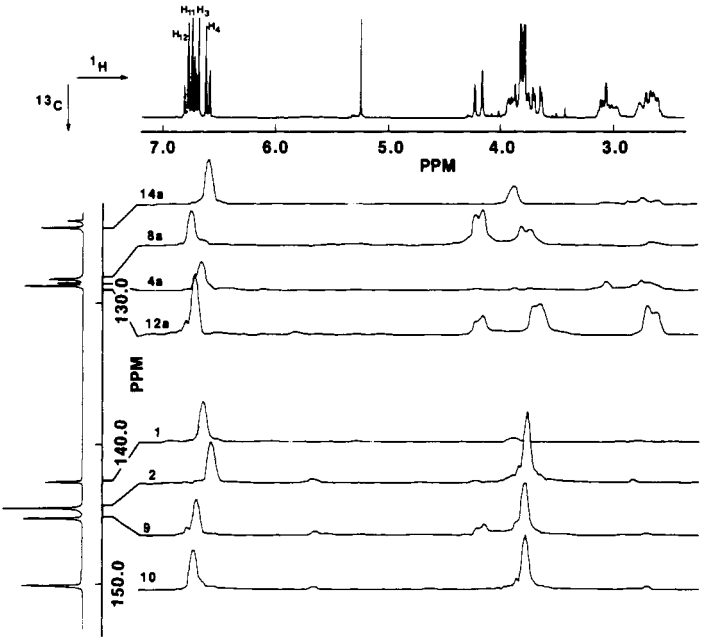


FIGURE 4. 2-D ^1H-^{13}C correlated experiment on caseanidine (**1**) using the three-bond coupling constant. Carbon assignments are given to the left of each trace.

possible to specify which of these chemical shifts corresponds to the axial and which to the equatorial protons.

In the second experiment (Figure 4), the aromatic quaternary carbons can be readily assigned since they are all coupled to one aromatic proton. They also experience long-range coupling (two or three bonds) due to an interaction with the protons located at benzylic positions or to those attached to methoxyl groups. Of the signals from oxygen-bonded sp^2 carbon atoms between 140 and 150 ppm, the one for C-1 is located furthest upfield. It is clearly the only aromatic C-0 signal not coupled to the methyl (OCH_3) proton but is coupled (3-bond) to H-3. Carbon atoms C-2, C-9, and C-10 are coupled to the protons of the methyl groups and also coupled (3-bond) to H-4, H-11, and H-12, respectively. The aromatic quaternary C-C signals between 120 and 130 ppm can be similarly assigned. Carbon 14a, located at 124.6 ppm, is the signal furthest upfield in this series and is coupled (3-bond) to H-4 and also coupled to H-14. Carbon nuclei at C-4a and C-8a, which resonate at 128.7 ppm and 121.0 ppm, are clearly coupled (3-bond) to H-3 and H-12, respectively, while carbon atom 12a at 128.9 ppm remains. Its coupling pattern is unusual since it interacts with the protons at C-13 and one or more of the protons located on C-8.

Clarkeanidine **2** was isolated from the "phenolic" ether extract using the procedure mentioned above (9). The molecular formula of **2** based on the high resolution mass spectrum was found to be $C_{19}H_{21}NO_4$. The following peaks were observed in the low resolution mass spectrum: *m/z* 327(58), 312(4.8), 310(5.8), 178(100), 163(12), 150(28.8), 135(12.2); and the ir spectrum had absorbances at 3537 cm^{-1} (OH stretch) and 2841 cm^{-1}, 2803 cm^{-1}, and 2756 cm^{-1} (weak Bohlmann bands). This base contained a similar set of *ortho* coupled aromatic protons (^1H-nmr spectrum) as **1**; however, in the case of **2**, they overlapped to a greater extent. The ^1H-nmr data are given in Table 1, and ^{13}C-nmr data, in Figure 2.

The fragmentation pattern in the mass spectrum of clarkeanidine (Scheme 1) indicates that an OH group is present in ring D in contrast to the methoxyl group of

caseanidine. The presence of two methoxyl groups and two OH peaks in the ^{1}H-nmr spectrum confirms this finding.

From a COSY experiment performed on **2**, it was obvious that each methoxyl group was *ortho* to an aromatic proton, which suggested that the C-9 methoxyl function in **1** had been replaced by an OH group in **2**. The ^{13}C-nmr spectrum also supports this assignment since the chemical shifts of C-3 and C-11 appear in the same resonance signal (108.89 ppm), and, likewise, the signals for C-4 and C-12 coincide (119.44 ppm). This indicates a very similar substitution pattern in rings A and D with respect to the aromatic protons. A quantitative ^{13}C-nmr spectrum confirmed this unexpected coincidence of peaks.

The structural assignment for rings A and D was confirmed by comparison of the ^{13}C-nmr signals for **2** with those of **1**. A 2-D ^{1}H-^{13}C correlated experiment on **2** using the three-bond coupling constant was employed to assign carbons 8a, 12a, 9, and 10. The sterochemistry of tetrahydroprotoberberines at the B/C ring junction has been the subject of much interest (14,15). From previous studies (15,16), it can be inferred that compounds **1** and **2** exist in solution as a mixture of *cis*- and *trans*-conformers at the B/C ring junction. This conclusion rests on the fact that the ^{13}C-nmr signals for C-6 in **1** and **2** (49.2 and 49.1 ppm, respectively) are upfield of the expected *trans* shift value of approximately 51.4 ppm (15); moreover, the Bohlmann bands are weak. The stereochemistry of **2** at C-14 is based on that of caseanidine (**1**) (10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Ci and eims were obtained on a Hewlett Packard 5985 gc/ms system. Uv spectra were recorded on a Hewlett Packard 8451A Diode Array Spectrophotometer using MeOH solutions of 0.05 mg/ml concentration. ^{13}C- and ^{1}H-nmr spectra were recorded at 250 MHz on a Bruker multiple-probe instrument and unless otherwise stated were run in $CDCl_3$ solution. Ir spectra were recorded on a Nicolet 10-MX spectrometer in $CHCl_3$ using NaCl cells. Tlc plates used were silica gel 60 F254, and column chromatography was performed on silica gel 60 (70-230 mesh). The optical rotations for both **1** and **2** were obtained in $CHCl_3$ (0.2%) on a Perkin-Elmer 241C Automatic Polarimeter.

The COSY spectra were recorded with the sequence $(90°\text{-}t_1\text{-}45°\text{-}t_2)_n$ (17). 512 Experiments were acquired with a size of 1K data points and a sweep width of 1250 Hz. Each dimension was zero-filled once before 2-D processing. The window used in the processing was a pure sine bell. The matrix was symmetrized before plotting.

The ^{1}H-^{13}C correlated experiments were recorded with the sequence given in reference 18. In the first experiment (Figure 3), the delay, Δ, was $1/(2^1J_{CH})=3.5$ ms. In the second experiment (Figure 4), Δ was $1/(2^3J_{CH})=50$ ms. One hundred twenty-eight experiments were acquired with a size of 2K data points and a sweep width of 6579 Hz. The sweep width in the first dimension is 1250 Hz. Each dimension was zero-filled once before 2-D processing. The window used in the processing was a sine bell shifted by $\pi/2$.

PLANT MATERIAL.—Leaves, stems, and flowers of *C. clarkei* Prain were collected by A.P. Brown and M.A. Rothera in August, 1984, while on a Cambridge expedition (3) to the small Himalayan region of Zanskar (15,000 ft) in the northwest Indian state of Jammu and Kashmir. An herbarium specimen has been deposited with the Royal Botanic Gardens, Kew, UK.

EXTRACTION AND PRELIMINARY FRACTIONATION.—Air-dried leaves, stems, and flowers of *C. clarkei* Prain (101.64 g) were extracted with MeOH for 7 days using a Soxhlet apparatus. After removal of the MeOH under reduced pressure, the residue (29.9 g.) was treated with 2% tartaric acid, and separated into alkaloid fractions according to the procedure of Tani *et al* (9).

ISOLATION OF THE ALKALOIDS.—Fractions obtained using the above procedure were analyzed on tlc for alkaloids by spraying with Dragendorffs reagent-Munier and Macheboeuf modification (19). The "non-phenolic" ether extract (320 mg) was chromatographed on silica gel and eluted with a solution of $CHCl_3$-Me_2CO (98:2) to yield 80 mg (0.078 % yield) of caseanidine (**1**). The "phenolic" ether extract (230 mg) was chromatographed on silica gel and eluted with $CHCl_3$-Me_2CO (85:15) to yield 50 mg (.049 % yield) of clarkeanidine (**2**).

CASEANIDINE.—Mp 169-170° (MeOH), literature 170° (10); $[\alpha]_D(CHCl_3)=-267$; ir ($CHCl_3$) 3530 cm^{-1} (OH stretch) 2838 cm^{-1}, 2758 cm^{-1} Bohlmann bands (12); uv λmax (MeOH, ε) 234 nm

(9679), 282 nm (5256); ^{1}H-nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (%) M+ (341,100), 326(7), 310(20), 178(8), 176(6), 164(82), 149(38); hrms calculated for $C_{20}H_{23}NO_4$ 341.1627; found 341.1614.

CLARKEANIDINE.—Mp 178-179° (iPrOH); $[\alpha]_D(CHCl_3)=-277$; ir ($CHCl_3$) 3537 cm^{-1} (OH stretch) 2841 cm^{-1}, 2803 cm^{-1}, 2756 cm^{-1} Bohlmann bands (12); uv λmax (MeOH, ε) 226 nm (11;200), 282 nm (3397); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (%) M+ (327,68), 312(4.8), 310(5.8), 178(100), 176(20), 163(12), 150(28.8), 135(12.2); hrms calculated for $C_{19}H_{21}NO_4$ 327.1470; found 327.1500. Since the $[\alpha]_D$ is negative for both **1** and **2**, both alkaloids possess the *S* configuration; the C-14 hydrogen atom is alpha (down).

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