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An alkaloid and a saponin from *Argemone mexicana*

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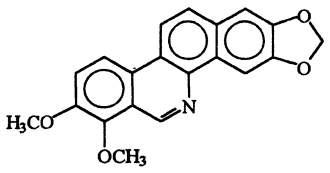
Argemone mexicana afforded norchelerythrine and sitosterol-β-D-glucoside. The separation was achieved by gravity column chromatography (dry packing). The structures of the compounds were elucidated by nmr spectroscopy and mass spectrometry.

Keywords. *Argemone mexicana*, norchelerythine, sitosterol-β-D-glucoside

ARGEMONE MEXICANA IS A SPINY HERBACEOUS ANNUAL SCATTERED throughout the Philippines. It is reported to possess a number of medicinal properties. The root decoction is prescribed for blennorhagia and vesicular calculus. It is used as an eyewash and mouthwash for toothache. The juice is used as a medicine for dropsy and jaundice. The seeds are used as laxative and the infusion of the leaves are used for coughs [1].

Earlier chemical investigations of various parts of the plant revealed a number of alkaloids [2-12] such as argemonine, norargemonine, berberine, chelerythrine, norchelerythrine, dihydrochelerythrine, sanguinarine, norsanguinarine; fatty acids, amino acids, flavonols, flavonolglycosides and chromones. We now report the isolation and structure elucidation of norchelerythrine (**1**) and sitosterol-β-D-glucoside (**2**) from the plant.



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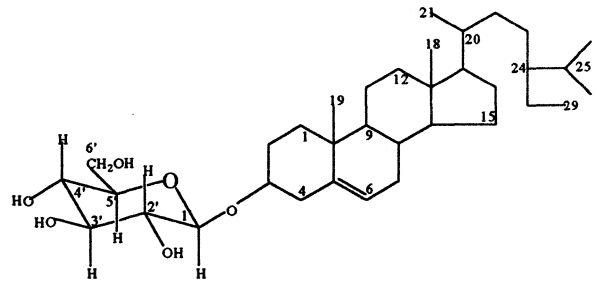
EXPERIMENTAL

Sample. *Argemone mexicana* was collected from Sinait, Ilocos Sur in May 1995 and February 1996. The plant was identified by the National Museum.

Extraction and Isolation. Air dried leaves (303 g) were extracted with chloroform (1.3 L) to afford a crude extract (13.5 g). The crude extract was treated with aq. $Pb(OAc)_2$ to precipitate the pigments [15]. The treated extract (1.75 g) was subjected to gravity column chromatography using increasing proportions of acetone in chloroform (10% increment) as eluent. The chloroform fraction was rechromatographed using chloroform as eluent to afford **1** (15 mg). The acetone fraction was rechromatographed in acetone to afford **2** (9 mg).

Instrumentation and General Procedures. The nmr spectra of **1** were recorded in $CDCl_3$ while the spectra of **2** were recorded in DMSO with the use of a GE 500 MHz nmr spectrometer. Eims was carried out on a JEOL D 100 mass spectrometer. Fractions were monitored by TLC and spots were visualized by spraying with vanillin:sulfuric acid, then warming. Melting points were obtained using the Fischer Johns melting point apparatus. The number of micronucleated polychromatic erythocytes were counted by the use of a Zeiss microscope.



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RESULTS AND DISCUSSION

The chloroform extract of the air-dried leaves of *Argemone mexicana* afforded norchelerythrine (**1**) and sitosterol-β-D-glucoside (**2**). Their structures were elucidated by nmr spectroscopy and mass spectrometry.

Compound **1** was isolated as yellow crystal. It gave a molecular ion peak at $[M^+] = 333$. An odd molecular weight indicates that **1** contains a nitrogen and therefore is an alkaloid. This may be expected since the plant is rich in alkaloids [2-12]. Cursory examination of the ^1H nmr spectrum of **1** indicated that it is aromatic based on the deshielded resonances of protons from δ 6.13 to δ 9.75 (Table 1). Two methoxy groups were attributed to the resonances at δ 4.13 (3H, s) and 4.08 (3H, s).

Table 1. Comparison of 500 MHz ^1H nmr spectral data of **1** in $CDCl_3$ with norchelerythrine [12]

H	1 (δ)	Norchelerythrine [12]
1	8.72 (1H, s)	8.75 (1H, s)
4	7.26 (1H, s)	7.26 (1H, s)
5	7.86 (1H, d, 8.74Hz)	7.86 (1H, d, 9.2 Hz)
6	8.35 (1H, d, 8.74Hz)	8.36 (1H, d, 9.2 Hz)
8	9.75 (1H, s)	9.78 (1H, s)
9	4.08 (3H, s)	4.10 (3H, s)
10	4.13 (3H, s)	4.17 (3H, s)
11	7.59 (1H, d, 9.09 Hz)	7.58 (1H, d, 9.0 Hz)
12	8.36 (1H, d, 9.09 Hz)	8.36 (1H, d, 9.0 Hz)
2,3 OCH2O	6.13 (2H, s)	6.16 (2H, s)

The ^{13}C nmr spectrum of **1** (Table 2) indicated twenty carbons, ten of which were deduced as quaternary based on their weak resonance intensities. Seventeen aromatic carbons were attributed to the deshielded resonances at δ 102.2 to δ 149.4. The deshielded resonances at δ 145.3, δ 148.3, δ 146.6 and δ 148.5 were attributed to aromatic carbons singly bonded to oxygens, while the resonance at δ 140.0 was assigned to an aromatic carbon singly bonded to nitrogen [12]. The more shielded resonances at δ 61.8 and δ 56.8 were assigned to two methoxy groups. The resonance at δ 101.3 was assigned to an OCH_2O [12].

The molecular ion peak corresponds to a molecular formula of $C_{20}H_{10}NO_4$ which is confirmed by the ^1H and ^{13}C nmr spectra of **1**. From the molecular formula, the index of hydrogen deficiency was fourteen. With nine double bonds deduced from the ^{13}C nmr spectrum, the remaining hydrogen deficiency could be attributed to five ring systems.

Table 2. 100 MHz ^{13}C nmr spectral data of **1** in $CDCl_3$

Chemical Shifts (δ)	Type of C
102.0, 104.4, 118.3, 118.4, 118.7, 120.0, 121.9, 127.1, 128.1, 129.2, 129.7	aromatic CH
145.3, 146.6, 148.3, 148.5, 149.4	aromatic C-O
140.0	aromatic C-N
101.3	OCH_2O
56.8, 61.9	OCH_3

These spectral data were compared to the structures of some alkaloids reported to have been isolated from *A. mexicana*. This led to the identification of **1** as norchelerythrine. Confirmatory evidence was the comparison of the ^1H nmr spectral data of **1** and norchelerythrine found in the literature (Table 1) [13]. The data matched in all essential respects. Furthermore, the mass spectra of **1** and that of norchelerythrine obtained from the library of mass spectra were similar. The base peak is the molecular ion peak indicating that **1** is stable. The peak at 318 (20%) resulted from the loss of CH_3 [M^+-CH_3], while the one at 290 (34.5%) resulted from the loss of CO [318-CO]. Another major peak at 275 (17.5%) resulted from the loss of the second CH_3 [290-CH_3]. The structure of **1** was further confirmed by its melting point (221°C) which is the same as that of norchelerythrine [12].

The ^1H nmr spectral data of **2** (Table 3) indicated resonances typical of a sterol. Six methyl groups were attributed to the resonances at δ 0.62 (3H, s), 0.74 (3H, d), δ 0.79 (3H, d), 0.76 (3H, t), δ 0.92 (3H, s) and 0.97 (3H, d) and an olefinic hydrogen at δ 5.30 (1H, s, br). The resonances at δ 2.86 (1H, m), δ 4.20 (1H, d, 9.1Hz), δ 3.05 (1H, m), δ 2.96 (1H, m), δ 3.10 (1H, m), δ 3.30 and δ 3.60 (1H, m) indicated a glycoside. The anomeric hydrogen was assigned to the doublet at δ 4.20. The J = 9.1 Hz is typical for an axial-axial coupling constant, hence, **2** is a β-D-glycoside. The resonances at δ 4.85 (3H, m) and δ 4.40 (1H, t) were assigned to the hydroxyl groups.

The COSY spectrum of **2** indicated the following isolated spin systems. The resonance of an anomeric hydrogen at δ 4.20 (H1', 1H, d) was coupled to the proton at δ 2.86 (H2', 1H, m), which was in turn coupled to the hydrogen at δ 3.05 (H3', 1H, m), which was further coupled to the proton at δ 2.96 (H4', 1H, m), which was also coupled to the hydrogen at δ 3.10 (H5', 1H, m), which was finally coupled to the methylene protons at δ 3.30 (H6', 1H, m) and δ 3.60 (H6', 1H, m). The resonance at δ 4.40 (1H, t) was assigned to a hydroxyl which showed small coupling with the methylene

Table 3. 500 MHz ^{1}H nmr spectral data of **2** in DMSO

Chemical Shifts (δ)	Type of H
5.3 (1H, s br)	olefinic H
4.85 (3H, m), 4.41 (1H, t)	OH
4.20 (1H, d)	anomeric H
3.60 (1H, m), 3.30, (1H, m), 3.10 (1H, m), 3.05 (1H, m), 2.96 (1H, m), 2.86 (1H, m)	carbinyl H's of glycoside
0.92 (3H, s), 0.86 (3H, d) 0.79 (3H, t), 0.78 (3H, d) 0.74 (3H, d), 0.62 (3H, s)	sterol methyl H's

protons at δ 3.30 (1H, m) and δ 3.60 (1H, m). The resonances centered at δ 4.85 (3H, m) were assigned to the other hydroxyl groups which showed small couplings with the carbinyl hydrogens at δ 2.86 (1H, m), δ 2.96 (1H, m) and δ 3.05 (1H, m). Another isolated spin system is indicated by the olefinic proton at δ 5.30 (s, br) which was coupled to the allylic protons at δ 1.90 and δ 1.92, which were in turn coupled to the protons at δ 1.46 and δ 1.15. Other isolated spin systems were not deduced due to overlapping resonances in the shielded region (δ 1.1- 2.0).

The ^{13}C nmr spectrum of **2** indicated resonances for a sterol (δ 11.8-61.1) which accounted for twenty-seven carbons. An olefin typical for a sterol is attributed to the resonances at δ 140.5 and δ 121.2. Thus, the sterol has twenty-nine carbon atoms. An anomeric carbon was assigned to the resonance at δ 100.0, while the resonances at δ 70.1-76.9 were attributed to the glycoside moiety. The ^{13}C nmr spectral data of **2** is summarized in Table 4.

Table 4. The 100 MHz ^{13}C nmr spectral data of **2** in DMSO

Chemical Shift, δ	Type of C
140.5, 121.2	olefinic C
100.8	anomeric C
76.9, 76.7, 76.7, 73.5, 70.1, 61.1	Carbinyl C
20.6, 22.6, 23.8, 25.4, 27.8, 28.7, 29.0, 31.4, 31.4, 33.3, 35.5, 36.2, 36.8, 38.6, 40.2 41.8, 45.1, 49.6, 55.4, 56.6	sterol C
19.7, 19.1, 18.9, 18.6, 11.8, 11.7	sterol methyl C

Correlation of the data obtained from nmr (^{1}H, ^{13}C, and COSY) indicated that **2** is a sterol glycoside. This was supported by the mass spectrum of **2** which gave the highest mass at m/e = 414 which was attributed to sitosterol [13]. The base peak at m/e = 396 is due to 414-H_2O. This is similar to the mass spectrum of sitosterol-β-D-glucoside [13]. The glycoside moiety gave the following fragmentations: 163 [M^+-$C_{29}H_{49}O$] and 145 [163-H_2O]. Literature search was conducted to compare the decomposition point of **2** with those of sitosterol glycosides. The decomposition point of sitosterol-β-D-glucoside is 285-290°C which is identical to that of **2** [13].

In summary, the chloroform extract of the air dried leaves of *A. mexicana* afforded norchelerythrine and sitosterol-β-D-glucoside by gravity column chromatography. Norchelerythrine was previously reported as constituent of *A. mexicana*, but this is the first report on the isolation of sitosterol-β-D-glucoside from the plant.

ACKNOWLEDGMENT

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