ARCUTININE, A NEW ALKALOID FROM *Aconitum arcuatum*

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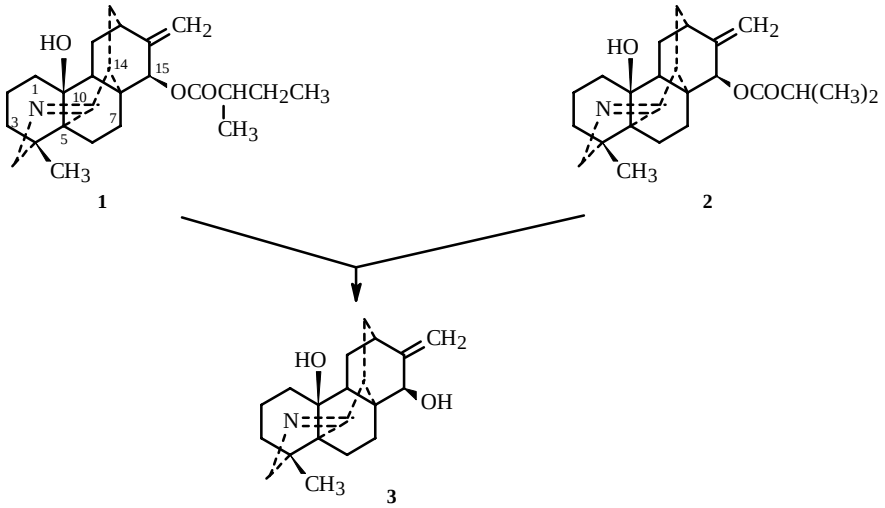
The structure of arcutinine isolated as a crystalline mixture with arcutine from the aerial part of Aconitum arcuatum *was established using ^{1}H and ^{13}C NMR, IR, and mass spectra and comparison with arcutine. Arcutinine, like arcutine, contains a C-5—C-20 bond instead of the usual C-10—C-20 bond in the C_{20}-diterpenoid carbon skeleton.*

Key words: *Aconitum arcuatum*, Ranunculaceae, new diterpenoid alkaloid arcutinine.

The isolation from the aerial part of *Aconitum arcuatum* Maxim. (Ranunculaceae) of a crystalline mixture containing the new alkaloid arcutine, for which the structure **1** was established by an x-ray structural analysis, has been reported [1]. Arcutine is a new C_{20}-diterpenoid alkaloid with a carbon skeleton that contains a C-5–C-20 bond instead of the C-10–C-20 bond typical of this class of alkaloids.

In continuation of this study we used PMR spectroscopy to show that the crystalline mixture from which arcutine was isolated [1] consists of arcutine (**1**) and a new alkaloid that we called arcutinine (**2**) in a 2:1 ratio.



The mass spectrum of the mixture of **1** and **2** contains peaks for the molecular ions of arcutine with *m*/*z* 397 and arcutinine with *m*/*z* 383. Very strong peaks for ions with *m*/*z* 313 (100%) and 295 (96%) are formed via loss from the arcutine molecular ion of fragments with *m*/*z* 84 $[M - COC_4H_8]^+$ and 102 $[M - COC_4H_8 - H_2O]^+$; from the arcutinine ion, with *m*/*z* 70 $[M - COC_3H_6]^+$ and 88 $[M - COC_3H_6 - H_2O]^+$. The elemental compositions of the ions were determined by high-resolution mass spectrometry (HRMS). These data indicate that arcutinine contains an isobutyric acid moiety whereas arcutine has a 2-methylbutyric acid moiety. Proton signals of the acyl fragments in the PMR spectra of **1** and **2** are described below.

The IR spectrum of the mixture of **1** and **2** has strong absorption bands for hydroxyl (3289 cm^{-1}), ester (1729 cm^{-1}), and N=C bonds (1645 cm^{-1}).

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TABLE 1. ^{13}C Chemical Shifts in a Mixture of Arcutine (**1**), Arcutinine (**2**), and Arcutinidine (**3**) in $CDCl_3$

C-atom	Mixture of **1** and **2**	**3**
1	33.34 33.52	32.71
2	20.56 20.60	20.16
3	31.24	30.12
4	39.63	40.19*
5	58.90	58.68
6	17.05	16.55
7	28.89 28.99	28.25
8	41.34	40.87*
9	43.97	42.53
10	75.33	75.06
11	26.27	26.00
12	35.83	35.35
13	32.55 32.70	31.85
14	37.47	37.10
15	74.08	73.64
16	150.80	155.31
17	110.38 110.57	108.55
18	23.50 23.63	23.43
19	73.83	73.26
20	185.63	186.0
21	175.80	-
22	41.83 34.77	-
23	27.26 (t) 19.51 (k)	-
24	12.10 19.58	-
25	17.22 -	-

*The signals may be switched.

The PMR spectrum of the mixture exhibits signals for a proton geminal to an acyl at 5.36 ppm (1H, t, J = 1.2 Hz), exomethyl protons at 4.94 and 4.85 ppm (1H each, dd, J = 1.2 Hz), and 19-methylene protons at 3.69 and 3.33 ppm (1H each, dd, J = 14.0 and 1.4 Hz). The lack of a signal for a proton geminal to a hydroxyl indicates that the hydroxyl in arcutinine, like in arcutine, is tertiary. A singlet for the protons of the methyl on C-4 occurs at 0.94 ppm. Signals for the 2-methylbutyric acid protons in arcutine appear at 0.88 (3H, t, J = 7 Hz, $CH_2\underline{CH_3}$) and 1.13 ppm (3H, d, J = 7 Hz, $CH\underline{CH_3}$), whereas those of isobutyric acid in arcutinine appear at 1.20 and 1.21 [3H each, d, J = 7 Hz, $CH(\underline{CH_3})_2$] and 2.63 ppm [1H, m, $\underline{CH}(CH_3)_2$].

The ^{13}C NMR spectrum has signals for the 20 C atoms of the diterpenoid skeleton and at 41.83, 27.26, 17.22, and 12.10 ppm for C atoms of the 2-methylbutyric acid in arcutine and at 34.77, 19.58, and 19.51 ppm for the C atoms of isobutyric acid in arcutinine. Analogous signals were found in ^{13}C NMR spectra of hetisine-type diterpenoid alkaloids, which have hydroxyls esterified by 2-methylbutyric [2-5] or isobutyric [5-7] acids.

Based on the above arguments and considering that the chemical shifts and multiplicity of the signals for the geminal acyl, exomethylene, 19-methylene, and 18-methyl protons are similar in arcutine and acrutinine, we proposed that arcutinine has the same heterocyclic skeleton as arcutine and the same position for the tertiary hydroxyl and ester on C-10 and C-15, respectively.

This hypothesis was confirmed by saponfying the mixture in methanol solution with base to form the aminoalcohol arcutinidine $C_{20}H_{27}NO_2$ (**3**). The peak for the molecular ion with *m*/*z* 313 in the mass spectrum of **3** is the base peak. Its PMR spectrum differs from that of the starting mixture by a strong-field shift of the signal for the gemacyl proton H-15α by 1.38 ppm and the lack of signals for protons of 2-methylbutyric and isobutyric acids.

These data indicate that arcutinine has structure **2**; arcutinidine, **3**.

The ^{13}C NMR spectrum of arcutinidine exhibits signals for 20 C atoms. Using DEPT, we found that these signals belong to one methyl, nine methylene, four methine, and six protonless C atoms. Signals for the C atoms were assigned based on their hybridization taking into account substituent effects on the chemical shifts and a comparison with model compounds.

Table 1 shows that signals at 186.00, 155.31, 75.06, and 58.86 ppm in the spectrum of **3** are assigned to C-20, C-16, C-10, and C-5; at 40.87 and 40.19 ppm, to C-4 and C-8 (or C-8 and C-4, respectively). Signals at 73.64 and 42.53 ppm belong to C-15 and C-9. Two other signals at 37.10 and 35.35 ppm and at 26.00 and 31.85 ppm belong to C-14 and C-12 and to C-11 and C-13, in analogy with known alkaloids that lack substituents on proximal C atoms in the bicyclo-[2.2.2]-octane system [8-10]. Signals at 16.55 and 20.16 ppm are assigned to C-6 and C-2, like for alkaloids with the atisine-azomethine skeleton [11]. The weak-field signal at 32.71 ppm is assigned to C-1 (β-effect of the C-10 OH); the strong-field signal at 28.25 ppm, to C-7 (γ-effect of the C-15 OH). Table 1 gives the assignments of the remaining signals.

Analogous signals are observed in the ^{13}C NMR spectrum of the mixture of **1** and **2** in which the shifts of most signals coincide (Table 1). Comparison of the spectra for **3** and the mixture of **1** and **2** showed that the chemical shifts of the diterpenoid C atoms are similar in the spectra of these compounds. The main difference is that the acyl substituent on C-15 shifts significantly the signal for C-16 (4.51 ppm) to strong field whereas that for C-17 (~2 ppm) is shifted to weak field. This is typical of diterpenoid alkaloids containing hydroxy and acyloxy groups, respectively, on C-15 [9, 12].

Thus, structure **2** proposed for arcutinine is confirmed by the ^{13}C NMR spectra of **1**, **2**, and **3**.

Among studied plants containing diterpenoid alkaloids, *A. arcuatum* is still the only one that produces compounds with a C-5–C-20 bond instead of the C-11–C-17 and C-10–C-20 bridging bonds common to all nor-, bisnor-, and diterpenoid alkaloids.

EXPERIMENTAL

General comments and the isolation of the crystalline mixture of arcutine (**1**) and arcutinine (**2**) (8 mg) have been published [1]. The IR spectrum of the mixture of **1** and **2** was obtained on a Perkin—Elmer System 2000 Fourier IR spectrometer; mass spectrum, in a MX-1310 instrument. The mass spectrum of **3** was taken in a Kratos MS 25 RF chromatograph-mass spectrometer (70 eV ionizing potential, 200°C source temperature, 120-150°C direct probe temperature, 100 μA collector current).

^{1}H and ^{13}C NMR spectra were taken on UNITY plus-400 (Varian) and WM 500 (Bruker) spectrometers in $CDCl_3$ with TMS internal standard.

Mixture of 1 and 2 (2:1 ratio). IR spectrum (KBr, ν, cm^{-1}): 3289, 3000, 2939, 2864, 1729, 1645, 1456, 1379, 1197, 1144, 1050, 1009, 974, 945, 900.

Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 397 $[M]^+$, arcutine (27), 383 $[M]^+$, arcutinine (18), 313 (100), 312 (33), 297 (17), 296 (27), 295 (96), 294 (24), 280 (20), 268 (23), 267 (36), 266 (26), 239 (21), 225 (24), 224 (40), 223 (17), 199 (23), 198 (69), 197 (27), 196 (28), 105 (36), 91 (37), 84 (31), 71 (40).

HRMS: Calc. for **1** $C_{25}H_{35}NO_3$ 397.2642, found 397.2616; for **2** $C_{24}H_{33}NO_3$ 383.2485, found 383.2443; for **3** $C_{20}H_{27}NO_2$ 313.2057, found 313.2041; for $C_{20}H_{25}NO$ 295.1951, found 295.1938.

Arcutinidine (3). A solution of arcutine and arcutinine (5 mg) in methanol (3 mL) was treated with methanolic base (3%, 5 mL). The reaction mixture was shaken at room temperature until the reaction was finished (30 h). The reaction was monitored by TLC. The solution was evaporated. The solid was chromatographed over silica gel (L 100/160, Czech Rep.) with elution by $CHCl_3$:ethanol (1:10) to give arcutinidine as an amorphous powder.

Mass spectrum, *m/z* (I_{rel}, %): 313 $[M]^+$ (100), 298 (8), 296 (20), 295 (10), 286 (15), 268 (9), 256 (15), 243 (8), 242 (15), 228 (6), 226 (6), 215 (8), 198 (19), 196 (6), 186 (10), 105 (8), 91 (12).

PMR (δ, ppm, J/Hz): 0.96 (3H, s, 4-CH_3), 3.46, 3.76 (1H each, dd, J = 16.0, 1.4, H-19α, H-19β), 3.98 (1H, t, J = 12.0, H-15α), 4.99, 5.07 (1H each, dd, J = 1.2, 2H-17).

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