STRUCTURE OF ACSINATINE

V. A. Tel'nov, S. K. Usmanova, and N. D. Abdullaev

UDC 547.944/945

The alkaloid acsinatine has been isolated from the roots of Aconitum leucostomum, and its structure has been established on the basis of spectral characteristics.

Acsinatine (I) was isolated some time ago from the roots of *Aconitum leucostomum,* and for it were proposed the composition $C_{21}H_{27}NO_4$ and the developed formula $C_{18}H_{23}(OH)(CO)(OCOCH_3)$ [1]. We have isolated lappaconitine, N-deacetyllappaconitine, lappaconidine, excelsine, sepaconitine, N-acetylsepaconitine [2] and acsinatine from this plant.

The IR spectrum of acsinatine contained the absorption bands of two hydroxy groups (3540 and 3450 cm^{-1}), of an ester carbonyl (1735 cm^{-1}) and of a terminal methylene group (3075, 1655, 895 cm^{-1}).

When (I) was acetylated with acetic anhydride in the presence of pyridine, an amorphous monoacetate (II) was obtained, while heating with acetic anhydride and *p*-toluenesulfonic acid gave a crystalline diacetate (III). Thus, acsinatine contains not only an acetoxy group but also two hydroxy groups: secondary and tertiary. On the basis of the facts given above, the elementary composition of (I) given previously [1] must be regarded as erroneous, and the formula of acsinatine be given as $C_{22}H_{29}NO_4$.

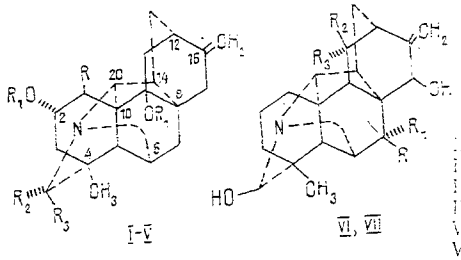
According to its composition and its PMR and ^{13}C NMR spectra, acsinatine belongs to the C_{20} group of diterpene alkaloids. On alkaline hydrolysis, (I) gave an amino alcohol — acsinatidine (IV) the IR spectrum of which lacked the absorption of a carbonyl group. The molecular masses of (I) and (IV) were 371 and 329, respectively. On the whole, the IR spectrum of (IV) was close to that of the alkaloid septentriosine (V), belonging to the hetisine group [3].

The ^{13}C NMR spectrum of acsinatine contained signals from 22 carbon atoms: 6 singlets, 7 doublets, 7 triplets, and 2 quartets. The assignment of these signals was made by a comparative analysis with the ^{13}C NMR spectra of ryosenamine [4], septentriosine (V), talatisine (VI) [5], ternatin (VII) [6], and acetyl derivatives of hetisine [7].

In the ^{13}C NMR spectra of (VI) and (VII), the signals of the C-19 carbon atoms appeared at 91.8 and 91.9 ppm, respectively, in the form of doublets, these carbon atoms bearing β-hydroxy groups, while in the ^{13}C NMR spectrum of (V) this signal appeared at 95.3 ppm (α-hydroxy group at C-19). The corresponding signal in the ^{13}C NMR of acsinatine was observed at 92.0 ppm. Consequently, there is a β-hydroxy group at C-19 in (I).

The presence in the PMR spectra of (I), (II), and (III) of signals at (ppm) 5.17 (1H, br.s), 5.25 (1H, m), and 5.31 (1H, m), and in the ^{13}C NMR spectrum of acsinatine of a doublet at 70.7 ppm and triplets at 31.8 and 47.8 ppm permits the conclusion that in acsinatine there is an α-acetoxy group at C-2 [4, 7, 8]. The tertiary hydroxy group in (I) may be present at the C-6, C-9, C-12, or C-14 carbon atoms. The detection of signals in the form of singlets at 78.8 and 50.4 ppm in the ^{13}C NMR spectrum of acsinatine permits the hydroxy group to be placed at C-9 [3, 4].

On the basis of what has been said above, acsinatine has the structure (I):



I–V VI, VII

I. $R=R_2=R_4=H$; $R_1=Ac$; $R_3=OH$
II. $R=R_2=R_4=H$; $R_1=Ac$; $R_3=OAc$
III. $R=R_2=H$; $R_1=R_4=Ac$; $R_3=OAc$
IV. $R=R_1=R_2=R_4=H$; $R_3=OH$
V. $R_1=R_3=R_4=H$; $R=R_2=OH$
VI. $R_1=R_3=R_4=H$; $R_2=OH$
VII. $R=R_2=H$; $R_1=OCOCH(CH_3)_2$; $R_3=OH$

Institute of Chemistry of Plant Substances, Academy of Sciences of the Uzbekistan Republic, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 409-411, May-June, 1993. Original article submitted October 5, 1992.

 0009-3130/93/2903-0346$12.50

The hetisine skeleton and the positions and configurations of the substituents are in full agreement with the two-dimensional PMR spectrum of acsinatine, the details of which will be published later.

It must be mentioned that the ester groups at C-19 in acsinatine monoacetate (III) readily underwent saponification during its purification on a column of alumina, giving the initial acsinatine.

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrophotometer, mass spectra on a MKh-1300 mass spectrometer fitted with a system for direct introduction into the ion source, PMR spectra on a BS-567 A (Tesla) instrument in deuterochloroform with HMDS as internal standard (values are given in the δ scale), and ^{13}C NMR spectra on a CFT-20 Varian spectrometer (in deuterochloroform, 0 — TMS).

Isolation of Acsinatine (I). The material from the mother solution of the total alkaloids from the epigeal part of *A. leucostomum* (250 g) after separation from lappaconitine [2] was dissolved in chloroform (1:10) and extracted with 5% sulfuric acid (16 × 80 ml). The acid solution was washed with ether (3 × 150 ml) and, in the presence of ice, was made alkaline fractionally with soda (pH 6, 7, 8, 10), the alkaloids being extracted each time with hexane, a mixture of hexane and ether, and ether (3 × 150 ml). After 48 h, the ethereal extracts obtained at pH 10 deposited a crystalline mixture of bases (13.67 g) showing three spots in TLC [KSK silica gel, chloroform—methanol (15:1)].

Part (5.9 g) of the crystalline mixture was chromatographed on a column of silica gel (KSK, 90-130 μm, 1:50) with elution by chloroform and mixtures of chloroform and methanol (1-5.0%) and the collection of 50-ml fractions.

After the solvent had been distilled off, fractions 4-8 were treated with methanol, and the crystals of acsinatine that deposited (1.6 g) were separated off and were recrystallized twice from ether—methanol (3:1), mp 251-253°C (0.92 g).

IR: 3540, 3455, 3075, 1735, 1655, 1455, 1442, 1430, 1380, 1348, 1330, 1260, 1248, 1200, 1175, 1160, 1147, 1118, 1093, 1076, 1060, 1025, 978, 950, 935, 915, 895, 880 cm^{-1}.

PMR: C—CH_3-18 — 1.01 (3H, s), $C\underline{H}_3CO$ — 1.98 (3H, s), 2.72 (1H, br.s), 3.45 (1H, br.s), 4.51 (2H, br.s, H-17 and H-19), 4.67 (1H, br.s, H-17), 5.17 ppm (1H, br.s, H-2-β).

Mass: M^+ 371 (45), 354 (13.6), 327 (9.0), 311 (100).

^{13}C NMR: C-1 — 31.8, C-2 — 70.7, C-3 — 37.8, C-4 — 42.2, C-5 — 55.1, C-6 — 60.8, C-7 — 29.6, C-8 — 42.1, C-9 — 78.8, C-10 — 50.4, C-11 — 39.0, C-12 — 36.9, C-13 — 34.3, C-14 — 43.9, C-15 — 31.7, C-16 — 152.1, C-17 — 104.3, C-18 — 23.0, C-19 — 92.0, C-20 — 70.1, $COCH_3$ — 169.6 and 21.7 ppm.

Acsinatidine (IV). Acsinatine (0.12 g) was heated in an aqueous methanolic solution of caustic soda (1.5%) in the water bath for 1 h, and the solvent was distilled off. The residue was dissolved in water and extracted with chloroform (6 × 50 ml). The extract was filtered and dried over anhydrous sodium sulfate, and the chloroform was distilled off. The residue was crystallized from aqueous acetone, giving acsinatidine (0.06 g) with mp 224-226°C.

IR: 3380, 3200, 3125, 1645, 1480, 1460, 1430, 1370, 1348, 1324, 1275, 1248, 1170, 1142, 1118, 1100, 1076, 1050, 980, 960, 935, 908, 885, 860 cm^{-1}.

Mass: M^+ 329 (3.7), 311 (100), 294 (13).

Acsinatine Monoacetate (II). Acsinatine (0.22 g) was dissolved in 6 ml of acetic anhydride and, after the addition of 1 ml of pyridine, the reaction mixture was left at room temperature for 48 h. The acetic anhydride and pyridine were eliminated in a rotary evaporator, the residue was dissolved in water, and the solution was made alkaline with soda and extracted with chloroform (15 × 50 ml). The extract was filtered and was dried over anhydrous sodium sulfate, and the chloroform was distilled off. The residue was treated with hot acetone, and the amorphous acsinatine acetate was separated off (0.17 g).

Mass: M^+ 413.

PMR: C—CH_3 (18) — 0.94 (3H, s), 2 CH_3CO at 2.07 and 2.09 (3H each, s), α-H-19 — 5.63 ppm (1H, s).

Acsinatine Diacetate (III). A mixture of acsinatine (0.16 g) and *p*-TSA (0.15 g) in 5 ml of acetic anhydride was heated in the water bath for one hour. Then the reaction mixture was poured into ice water and, after alkalinization to pH 10, it was extracted with chloroform (5 × 50 ml). The extract was filtered and was dried over anhydrous sodium sulfate, and the solvent was distilled off. The residue (0.18 g) was treated with petroleum ether, and the crystals that deposited were recrystallized from petroleum ether—diethyl ether (1:1). This gave 0.12 g of (IV) with mp 195-198°C.

Mass: M^+ 455 (1.5), 396 (100), 354 (5.3), 294 (6.0).

PMR: C—CH_3-18 — 0.9 (3H, s), $C\underline{H}_3CO$ — 1.88 (3H, s), 2 $C\underline{H}_3CO$ — 2.06 (6H, s), α-H-19 — 5.69 ppm (1H, s).

REFERENCES

1. T. F. Platonova, A. D. Kuzovkov, and P. S. Massagetov, *Zh. Obshch. Khim.*, **28,** 258 (1958).
2. V. A. Tel'nov, M. S. Yunusov, N. D. Abdullaev, and M. G. Zhamierashvili, *Khim. Prir. Soedin.*, 556 (1988).
3. B. S. Joshi, H. K. Desai, S. W. Pelletier, E. M. Holt, and A. S. Aasen, *J. Nat. Prod.*, **51,** 265 (1988).
4. S. Sakai, K. Tamaguchi, J. Tamamoto, K. Hotoda, T. Okazaki, N. Aomi, J. Haginiya, and T. Okamoto, *Chem. Pharm. Bull.*, **31,** 3338 (1983).
5. Z. Karimov and M. G. Zhamierashvili, *Khim. Prir. Soedin.*, 335 (1981).
6. V. M. Matveev, Author's Abstract of Candidate's Dissertation [in Russian], Dushanbe (1988).
7. J. A. Glinski, B. S. Joshi, Q. P. Jiang, and S. W. Pelletier, *Heterocycles,* **27,** 185 (1988).
8. M. G. Reinecke, D. S. Minter, D. C. Chen, and W. M. Yan, *Tetrahedron,* **42,** 6621 (1986).