Chem. Pharm. Bull. 20(9)1940—1944(1972)

UDC 547.99.02 : 581.192 : 615.322.011.5

The Structure of Cimifugin, a New Bitter Principle from *Cimicifuga simplex* Wormsk.[1]

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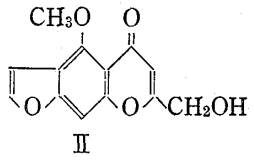
(Received February 22, 1972)

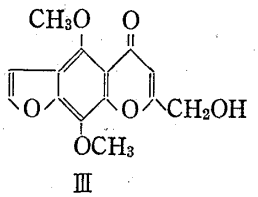
Khellol, ammiol, caffeic acid dimethyl ether, and cimifugin, a new bitter cumarin derivative have been isolated from the aqueous extract of the rhizome of *Cimicifuga simplex* Wormsk. (Ranunculaceae). The structure and the absolute configuration of cimifugin have been shown as IV and XI by chemical and physical methods.

Cimicifuga simplex Wormsk. (Ranunculaceae) is a plant indigenous to Asia and its rhizoma has been used for many years as an antipyretic and a spasmolytic in Japanese folk medicine.

The water extract of the rhizoma yielded a bitter resinous fraction which on chromatography gave a 0.064% of yield of a crystalline component, mp 105—106°, $[\alpha]_D$+79.2° ($CHCl_3$), besides the known compounds, caffeic acid dimethyl ether (I), khellol (II),[3] and ammiol (III).[4] This compound which is responsible for the bitter taste of the plant has been named cimifugin.



II



III

Cimifugin analyzed for $C_{16}H_{18}O_6$, a formula which was confirmed by high-resolution mass spectrum molecular ion peak 306.316 (Calcd. 306.320). The ultraviolet absorption spectrum showed maxima at 229.3 nm (infl.) (log ε 3.85), 250.5 nm (infl.) (log ε 3.75) and 293 nm (log ε 4.15) which was very similar to that of visamminol methyl ether.[5] Addition of alkali did not shift the ultraviolet (UV) maxima of cimifugin. This together with the negative ferric chloride color test indicated the absence of a free phenolic group. The infrared spectrum displayed bands at 3367 cm^{-1} (hydroxyl), 1661 cm^{-1} (conjugated carbonyl), 1623 cm^{-1} (double bond) and 1585 cm^{-1} (aromatic).

Cimifugin gave an oily monoacetate (V), $C_{18}H_{20}O_7$, on mild acetylation. V exhibited a free hydroxyl absorption at 3509 cm^{-1} (film) in the infrared (IR) spectrum and formed a diacetate (VI), $C_{20}H_{22}O_8$, under more drastic acetylating conditions. The diacetate (VI) showed no hydroxyl absorption in the IR spectrum. In agreement with this, the nuclear magnetic resonance spectra (Fig. 2) of cimifugin, its monoacetate (V) and diacetate (VI) indicated that cimifugin contained a primary and a tertiary alcoholic hydroxyl groups; this

1) This paper is Part VIII of "Studies on the Constituents of *Cimicifuga spp.*" For previous paper see T. Takemoto G. Kusano, and N. Yamamoto, *Yakugaku Zasshi*, **90**, 68, (1970).
2) Location: *Aoba-yama, Sendai.*
3) E. Späth and W. Gruber, *Chem. Ber.*, **74**, 154 (1941).
4) G. Seitz, *Arch. Pharm.*, **287**, 79 (1954).
5) W. Bencze and H. Schmid, *Experientia*, **10**, 12 (1954).

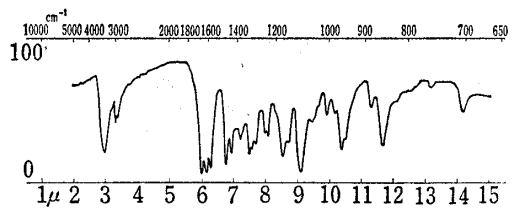


Fig. 1. IR Spectrum of Cimifugin (IV) in KBr Tablet

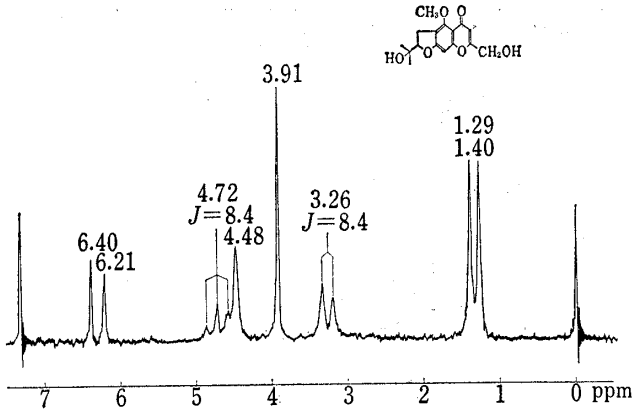


Fig. 2. NMR Spectrum of Cimifugin (IV) in $CDCl_3$

was evident from the characteristic downfield shift (0.41—0.46 ppm) of the α-methylene protons on conversion of the alcohol to its acetates[6] (see Table I). In the nuclear magnetic resonance (NMR) spectrum of cimifugin, two tertiary methyl groups appeared at 1.29 and 1.40 ppm, indicating that the methyl groups were bonded to the tertiary hydroxyl of the isopropyl side chain. The methylene group, a doublet at 3.26 ppm (J=8.4 Hz), and one proton, a triplet at 4.72 ppm (J=8.4 Hz), with typical A_2X splitting, indicate the partial structure -O-CH-CH_2-C-. A sharp singlet at 3.91 ppm was due to the methoxy group. The α-methylene protons of the primary alcohol and two olefinic protons showed up at 4.48, 6.21, and 6.40 ppm, respectively.

Table I. The Chemical Shifts of Compound IV, V, and VI in $CDCl_3$

	$(CH_3)_2$	-CH_2-	-OCH_3	-CH_2OR^2	H, R^1O, O	Olefinic H	Ring H	OAc
IV	1.29 1.40	3.26 (d, J=8.4)	3.91	4.48 ($J\lesssim1$)	4.72 (t, J=8.4)	6.21	6.40	—
V	1.26 1.36	3.30 (d, J=8.5)	3.96	4.95[a]	4.77 (dd, J=8.5)	6.19 (t, $J\approx1$)	6.56	2.18
V[b]	1.14 1.29	3.18 (d, J=8.5)	3.82	4.55	4.45 (t, J=8.5)	6.09	6.34	1.70
IV	1.50 1.56	3.25 (d, J=8.7)	3.92	4.89	5.06 (t, J=8.7)	6.14	6.50	1.98 2.17

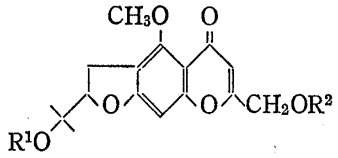
a) unresolublet *b*) in C_6H_6

When cimifugin was oxidized with chromic acid, acetone was formed and was identified as the 2,4-dinitrophenylhydrazone.

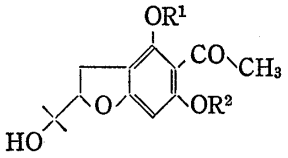
Cimifugin on heating with aqueous alkali gave a phenolic compound, mp 89.5—90.5°, $C_{14}H_{18}O_5$, molecular ion peak 266, $[\alpha]_D$+84.8° ($CHCl_3$), UV λ_{max}^{EtOH} 218.5 nm (log ε 4.2), 234.6 nm (log ε 3.7), *ca.* 240 nm (infl.) (log ε 3.6), 290 nm (log ε 4.2) and *ca.* 325 nm (infl.) (log ε 3.7). The IR spectrum of this phenolic compound showed the absence of the conjugated carbonyl group of cimifugin. The NMR spectrum of the phenol revealed the presence of an acetyl group at 2.62 ppm. From the physical and spectral properties it was concluded that this phenol was identical with 2-hydroxyisopropyl-4-methoxy-5-acetyl-6-hydroxy-cumaran (VIIa).[7] The mass spectrum (Chart 1) of the phenol is consistent with this assigned struc-

6) L.M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London 1959, p. 55.
7) W. Bencze, J. Eisenbeiss, and H. Schmid, *Helv. Chim. Acta*, **39**, 923 (1956).

ture. The another possible structure (VIIb) could be ruled out because the phenol show the negative Gibbs colour test.



Ⅳ: $R^1=R^2=H$
Ⅴ: $R^1=H$, $R^2=Ac$
Ⅵ: $R^1=R^2=Ac$



Ⅶa : $R^1=Me$, $R^2=H$
Ⅶb : $R^1=H$, $R^2=Me$
Ⅷ : $R^1=R^2=Me$

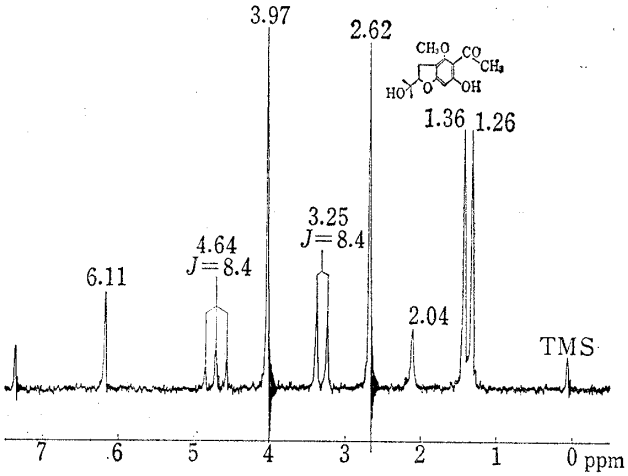


Fig. 3. NMR Spectrum of Compound VIIa in $CDCl_3$

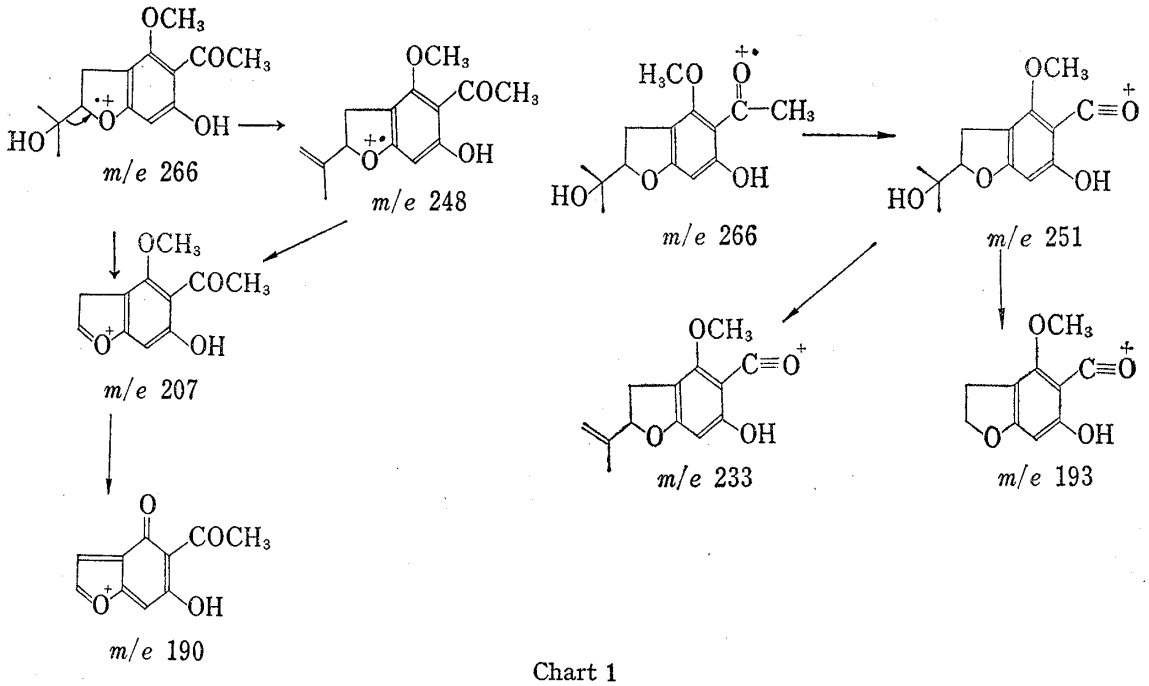


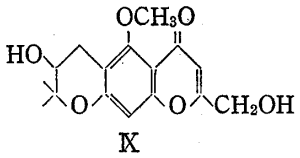
Chart 1

Based on these findings cimifugin is best represented by structure IV. Another possible structure IX may be rejected, since methyl ether (VIII) of the phenol VIIa did not undergo Jones oxidation, the mass spectra of cimifugin and phenol VIIa showed mass peaks due to loss of M-59, corresponding to elimination of the hydroxyisopropyl group, and acetates V and VI showed the resonable downfield shifts of two tertiary methyl groups in the NMR spectra.

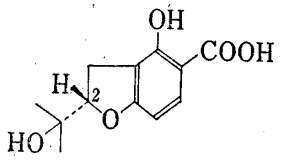
The absolute configuration of cimifugin was determined by molecular rotation. Comparison of the molecular rotation of 2-hydroxyisopropyl-4-methoxy-5-acetyl-6-hydroxy-cumaran, $[M]=+225.8°$ and (+)-2-hydroxyisopropyl-4-hydroxy-5-carboxy-cumaran (X), $[M]<+340°$, of known absolute configuration (2S)[8] showed approximately the same value.

8) B.E. Nielsen and J. Lemmich, *Acta Chem. Scand.*, **18**, 2111 (1964).

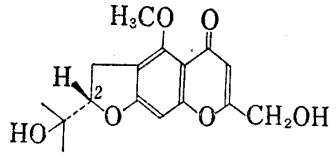
This allows assignment of the absolute configuration of VIIa as the S-configuration. Consequently, the absolute configuration of cimifugin was represented by the formula XI.



IX



X



XI

It is known that the furochromon derivatives possess marked spasmolytic action.[9] The usage of the rhizoma of *C. simplex* Wormsk for a spasmolytic is reasonable on account of pharmacological effect of the ingredients, khellol and ammiol.

Cimifugin exhibited activity as a central nervous system depressant.[10] Recently it has been reported that a new triterpenoid glycoside named cimicifugoside isolated.[11]

Experimental[12]

Isolation of Caffeic Acid Dimethyl Ether (I), Khellol (II), Ammiol (III) and Cimifugin (IV)——Air dried rhizoma *Cimicifuga simplex* Wormsk. (Ranunculaceae) (13.3 kg), collected at Gumma Prefecture, was extracted with cold MeOH three times and the combined MeOH extract was concentrated *in vacuo*. The brown viscous oil was shaken with petrol. ether to remove fatty material. The residue was dissolved in hot water and insoluble material was removed by filtration through a Celite layer. The resulting aqueous solution was treated with $Pb(AcO)_2$ aq and then basic lead acetate aq. The aqueous layer was extracted with $CHCl_3$. The $CHCl_3$ extract left a crystalline substance on removal of the solvent. Recrystallization from MeOH gave cimifugin (IV) as colorless needles, mp 102—102.5°. Yield: total 8.51 g (0.064%). Analytical sample was purified twice by recrystallization. mp 107—109°. *Anal.* Calcd. for $C_{16}H_{18}O_6 \cdot 2H_2O$: C, 60.94; H, 6.97. Found: C, 61.08; H, 5.85. M^+ 306.320. $[\alpha]_D^{28}$ +79.2±1.0° (c=0.8; $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 229.3 (infl.) (3.94), 244 (infl.) (3.85), 250.5 (infl.) (3.75) and 293 (4.15). IR ν_{max}^{KBr} cm^{-1}: 3367, 1661, 1623, 1585, 1095, 855.

The mother liquor of IV was evaporated under the reduced pressure to leave an oily residue which showed five spots on TLC. The mixture was chromatographed on a silica gel column (30×320 mm), the column was eluted with a mixture of $CHCl_3$–MeOH (97.5:2.5, 600 ml, 95:5, 400 ml) and fractionated into 5-ml portions.

Fractions 58—60 were pooled and evaporated to give 100 mg of a crystalline residue. Recrystallization from ether gave 80 mg of caffeic acid dimethyl ether (I) as colorless needles or plates, mp 175—176°. *Anal.* Calcd. for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.76; H, 5.88. M^+ 208. NMR ($CDCl_3$) ppm: 3.91 (6H, 2×C$\underline{H}_3$O-), 6.31 (1H, d, J=15.7 Hz, olefinic proton), 6.7—7.2 (3H, ring protons), 7.65 (1H, d, J=15.7 Hz, olefinic proton).

Fractions 77—83 were collected and evaporated to give a crystalline material. Recrystallization from acetone gave khellol (II) as colorless needles, mp 175—176° (lit,[5] mp 178°). M^+ 246. NMR (CD_3OD+$CDCl_3$) ppm: 4.17 (3H, -OC$\underline{H}_3$), 4.50 (2H, -C$\underline{H}_2$OH), 6.30 (1H, J<1Hz, α proton of $\alpha\beta$-unstaurated ketone), 7.16 (1H, d, J=2.3 Hz, furan ring proton), 7.28 (1H, benzene ring proton), 7.76 (1H, d, J=2.3 Hz, furan ring proton).

Fractions 90—95, on evaporation, left a crystalline residue. Recrystallization from acetone gave ammiol (III) as colorless needles, mp 205—207° (lit.,[6] 211°). *Anal.* Calcd. for $C_{14}H_{12}O_6$: C, 60.87; H, 4.38. Found: C, 60.36; H, 4.55. M^+ 276. NMR (CD_3OD+$CDCl_3$) ppm: 4.06 (3H, -OC$\underline{H}_3$), 4.19 (3H, -OC$\underline{H}_3$),

9) G. Illing, *Arzneim. -Forsch.*, 7, 497 (1957); K. Uhlenbroock and K. Mulli, *ibid.*, 7, 166 (1957).
10) We thank the Lederle Laboratories, New York and Dr. J.J. Denton in this Laboratories for carrying out pharmacological tests.
11) G. Kusano, S. Matsumoto, and T. Takemoto, 1 lth Symposium on the Chemistry of Natural Products (Kyoto), Abstract, 1967, p. 338.
12) M. ps were determined on a Büchi apparatus and not corrected. NMR spectra were recorded on a Hitachi H-60 and a Varian A-60 spectrometers. Chemical shifts of deuteriochloroform are recorded as ppm with tetramethylsilane as internal standard. A Hitachi EPI and EPS-3 spectrometers for infrared and ultraviolet spectra. Optical rotations were obtained on a Perkin-Elmer polarimeter Model 141. Mass spectra were determined with a AEI-MS 9 mass spectrometer.

4.58 (2H, -C$\underline{H}_2$OH), 6.37 (1H, benzene ring proton), 7.07 (1H, d, J=2.5 Hz, furan ring proton), 7.73 (1H, d, J=2.5 Hz, furan ring proton).

Fractions 143—170 included cimifugin.

Cimifugin Monoacetate (V)——To a soln of 300 mg of IV in 2 ml of dry pyridine was added 1 ml of acetate anhydride. After standing for 10 hr at room temperature the reagents were evaporated under reduced pressure. The crude acetates were purified over silica gel column (10×250 mm). The eluates with $CHCl_3$–AcOEt (9:1), which were homogenous (*Rf*, 0.36; AcOEt) were pooled and evaporated *in vacuo*. Colorless oil showed one acetyl group in NMR. Found: M$^+$ 348; $C_{18}H_{20}O_7$. requires: 348. IR $\nu_{max}^{liq.}$ cm^{-1}: 3509, 1748, 1661, 1613, 1582, 1217, 1087.

Cimifugin Diacetate (VI)——A soln of IV was allowed to react with acetic anhydride in dry pyridine at 90° for 8 hr. The reaction mixture was worked up as the usual fashion. The remaining oil was chromatographed over silica gel using successively $CHCl_3$ and $CHCl_3$–AcOEt (9:1) as eluting solvents. The eluates having *Rf* 0.60 were collected and evaporated *in vacuo*. Homogeneous diacetate was obtained as colorless oil. Found: M$^+$ 390; $C_{20}H_{22}O_8$·requires: 390.

Chromic Acid Oxidation of IV——To a solution of 100 mg of IV in 5 ml of hot water was dropwise added 2 ml of chromic acid solution (prepared from 6 g of $K_2Cr_2O_7$ and 8 g of conc. H_2SO_4 in 27 ml of water) under the nitrogen atmosphere. The reaction mixture stirred for 30 min at 60° and then the volatile compound was distilled off and collected in a trap cooled in liquid N_2. A ethanolic solution of 2,4-dinitrophenylhydrazine and few drops of conc HCl were added to the distillate and the mixture was allowed to stand to deposit a crystalline mass. Recrystallization from EtOH gave yellow crystal, mp 128°, which is identical with authentic acetone 2,4-dinitrophenylhydrazone.

Degradation of IV with Alkali to 2-Hydroxyisopropyl-4-methoxy-5-acetyl-6-hydroxy-cumaran (VIIa) A soln of IV (1.1 g) in 300 ml of 1% $Ba(OH)_2$ aq was refluxed under N_2 atmosphere for 15 min. After being cooled, the reaction mixture was acidified with HCl and then extracted with ether. The combined ether layer concentrated to yield colorless crystal. Recrystallization from MeOH aq give 850 mg of prisms, mp 89—90.5°. *Anal.* Calcd. for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 63.26; H, 6.32; M$^+$ 266. $[\alpha]_D^{28}$ +84.80° (*c* 1.06; $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 218.5 (4.2), 234.6 (3.7), ca. 240 (infl.) (3.6), 290 (4.2) and *ca.* 325 (infl.) (3.7). IR ν_{max}^{KBr} cm^{-1}: 3425, 1623, 1690, 1575, 1245, 825. NMR ($CDCl_3$) ppm: 1.26 and 1.36 (=(C$\underline{H}_3$)$_2$), 2.04 (OH), 2.62 (-COC$\underline{H}_3$), 3.25 (d, J=8.4 Hz, -CH-C$\underline{H}_2$-), 3.97 (-OC$\underline{H}_3$), 4.64 (t, J=8.4 Hz, -C$\underline{H}$-CH$_2$-), 6.11 (ring proton). The same results were obtained using 10% or 20% KOH aq instead of 1% $Ba(OH)_2$ aq. The comparison of *Rf* values on TLC (silica gel; $CHCl_3$–MeOH (96:4)) and the mixed melting point showed this material is identical with an authentic sample prepared from O-methyl visamminol by alkali degradation.[13]

VIIa Methyl Ether (VIII)——To a soln of 200 mg of VIIa in 1 ml of 20% KOH aq was added 0.5 ml of $(Me)_2SO_4$ under the mechanical agitation. After stirring for 30 min, 1 ml of 20% KOH aq and 1 ml of $(Me)_2SO_4$ added and then the reaction mixture was heated on steam bath for 1 hr. This procedure was repeated until all the starting material has been exhausted. Finally, the reaction mixture was acidified with HCl and then taken up in ether. Ether layer was washed with H_2O, dried and evaporated to give yellow oil. Purification over Al_2O_3 column chromatography give methyl ether (VIII) as colorless oil. Found: M$^+$ 280; $C_{15}H_{20}O_5$. requires: M 280. NMR ($CDCl_3$) ppm: 1.23 and 1.28 (=(C$\underline{H}_3$)$_2$), 2.40 (-COC$\underline{H}_3$), 2.82 (OH), 3.19 (d, J=8.7 Hz, -CH-C$\underline{H}_2$), 3.69 and 3.81 (2×-OC$\underline{H}_3$), 4.57 (t, J=8.7 Hz, -C$\underline{H}$-CH$_2$-), 6.11 (ring proton).

Acknowledgement The authors wish to thank Dr. John W. Daly, Laboratory of Chemistry, NIAMD, National Institutes of Health, U.S.A., for peak matching on the high-resolution mass spectrum.

13) We thank Prof. Dr. H. Schmid, Zürich University, for identification of compound VIIa.