[Chem. Pharm. Bull. 24(1) 176—178 (1976)]

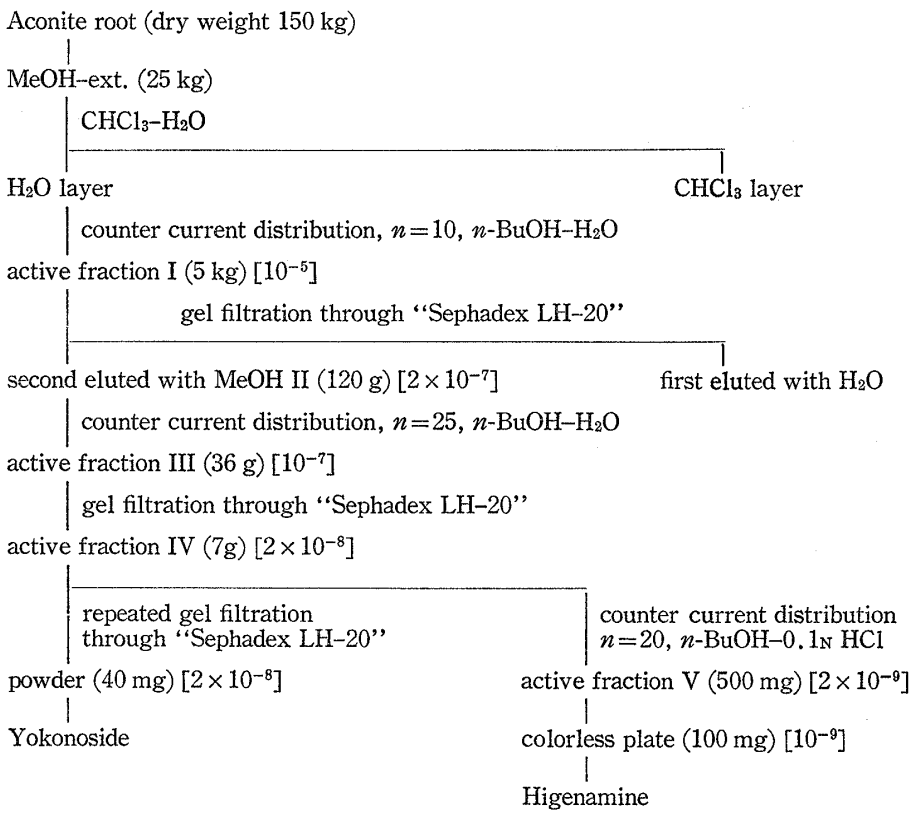
UDC 547.94.02.05.09 : 615.221.076.9 : 581.192

Studies on Cardiac Principle of Aconite Root

The chemical structure of Higenamine, cardiac principle isolated from *Aconitum japonicum* THUMB., was identified as *dl*-demethyl coclaurine.

Aconite root (Bushi in Japanese), *Aconitum japonicum* THUMB. has long been used as one of the most important herbs of Chinese medicine as a heart stimulant, diuretic agent and anodyne. Previous work on aconite root has been mostly focussed on diterpene alkaloids as toxic principles. Pharmacological studies relating to cardiac activity have been reported by Yakazu[1] and Lao.[2] However, any detailed chemical study on the cardiac principle has not yet been reported.

This communication deals with isolation and identification of the principle predicted by Yakazu. In the isolation process, the Yagi-Hartung method using frog's heart was found to be useful for pharmacological measurement of cardiac activity of the material. The material was fairly unstable, especially in basic medium, and was tightly adsorbed in charcoal, alumina, silica gel, cellulose powder and "Sephadex" G-types, indicating that these agents are not useful for the separation. Isolation of the material was finally achieved, however, by the limited combination of gel filtration through "Sephadex LH-20" and counter current distribution. The procedures are summerized in Fig. 1.

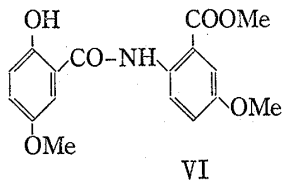


( ) indicates yield, [ ] indicates minimum active concentration

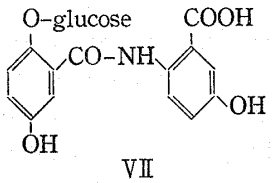
Fig. 1

1) S. Yakazu, *J. Pharm. of Japan*, 54, 895 (1958).
2) M. Lao, *Acta Pharmaceutica Sinica*, 13, 195 (1966).

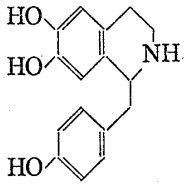
Aconite roots (150 kg) were ground and extracted with refluxing methanol. The water soluble portion of the methanol extracts was separated by counter current distribution using the solvent system *n*-buthanol and water, to give fraction I (5 kg) (M.A.C.,[3] one hundred thousand fold dilution). Gel filtration of fraction I through a "Sephadex LH-20" column was accomplished firstly with water and then with methanol as elution solvents. Active fraction II (120 g) eluted with methanol (M.A.C., five million fold dilution) was further purified by counter current distribution using the solvents mentioned above, yielding active fraction III (36 g) (M.A.C., ten million fold dilution), as a brown syrup. Fraction III was further subjected to repeated purification by the gel filtration technique mentioned above, yielding a pale yellow product stimulating frog's heart on fifty million fold dilution. This product, however, appeared not to contain the active component since repeated purification of fraction IV resulted in no marked increase in activity (M.A.C., fifty million fold dilution). The product was designated Yokonoside (mp 205—210°), a β-glucoside very soluble in water. Methylation of Yokonoside and subsequent acid hydrolysis, gave a 40% yield of a trimethyl derivative of aglycon as crystalline needles. The structure (VI) was determined by comparison with an authentic sample, which suggested that Yokonoside possessed structure VII.[4] Compound VII was independently synthesized to verify that it is the active component. However synthetic Yokonoside did not show any activity,[5] indicating that the real active species might be contained in the Yokonoside fraction as an impurity. The separating procedure was then more closely studied. Since the active fraction IV and the solid yellow product both showed the same activity, fraction IV was reinvestigated and found to contain a large proportion of Yokonoside. In order to remove the glucoside, active fraction IV (7 g) was subjected to counter current distribution using a solvent system of *n*-butanol and 0.1N-hydrochloric acid, affording active fraction V (500 mg) (M.A.C., five hundred million fold dilution). On standing overnight, a crystalline material (100 mg) separated from fraction V as colorless plates. On repeated crystallization the activity remained constant throughout, thus leading to the conclusion that these crystals represent the true cardiac species of the aconite root. This product has been designated as Higenamine [mp 260° (d), $\alpha_D^{20}=0°$].



VI



VII



VIII

Higenamine was identified as 1-(*p*-hydroxy-benzyl)-6,7-dihydroxy-1,2,3,4-tetrahydro isoquinoline (*dl*-demethyl coclaurine) VIII by comparison with an authentic sample.[6] Demethyl coclaurine is very unstable in basic medium as reported by Koshiyama,[7] having a half life at room temperature of 45 minutes at pH 8.0 and less than 10 minutes at pH 9.0. Yokonoside may play a part in the stabilization of Higenamine both in the plant and also during the isolation procedure firstly as a result of salt formation and secondly by acting as antioxidant, since Yokonoside possesses both a carboxylic acid function and the structural elements of a hydroquinone. Demethyl coclaurine has already been synthesized by Yamaguchi,[6] and (+)-demethyl coclaurine has been isolated by Koshiyama, *et al.*, from the embryo of *Nelumbo*

3) Minimum active concentration.
4) T. Kosuge and M. Yokota, 16th Symposium on the Chemistry of Natural Products, Osaka, Japan, Symposium paper, p. 335, 1972.
5) T. Kosuge and M. Yokota, presented at 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, 1974.
6) H. Yamaguchi, *Yakugaku Zasshi*, **78**, 692 (1958).
7) H. Koshiyama, H. Ohkuma, and H. Kawaguchi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2564 (1970).

nucifera.[7] (+) And (−)-demethyl coclaurine were obtained by hydrolysis of (+) and (−)-7-O-benzyl coclaurine,[8] respectively. (−)-Demethyl coclaurine showed far greater cardiac activity than (+)-demethyl coclaurine in the frog's heart test. This represents the first reported instance in which the active (−) form of demethyl coclaurine has been found in natural products such as Higenamine, which also contained the less active or the inactive (+)-form.

Further studies on the biological activities of Higenamine on Mammalia are at present being carried out by a Pharmacologist.

Acknowledgement The authors are deeply indebted to Prof. T. Okamoto of the University of Tokyo for his encouragement at all times and also to Dr. H. Zenda of their laboratory for his kind help.

Shizuoka College of Pharmacy
2-2-1, Oshika, Shizuoka

Takuo Kosuge
Masami Yokota

Received August 28, 1975

8) T. Kametani, K. Sakurai, S. Kano, and H. Iida, *Yakugaku Zasshi*, **87**, 822 (1967).

[Chem. Pharm. Bull. 24(1) 178—180 (1976)]

UDC 547.918.02.05 : 581.192

The Structure of Arjungenin. A New Sapogenin from *Terminalia arjuna*

The structure of a new sapogenin, arjungenin, isolated from *Terminalia arjuna* was shown to be 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oic acid (I).

The isolations and structure determinations of β-sitosterol, ellagic acid, D(+)-mannitol, (+)-leucocyanidin, (+)-leucodelphinidin, oleanolic acid, arjunic acid, arjunolic acid, and arjunetin from *Terminalia arjuna* have been reported.[1a,b,c,2] We have recently examined the methanol extract of the bark of the plant and isolated a new sapogenin which we named arjungenin. In this communication, we wish to report evidence leading to the structure I for arjungenin.

Arjungenin, mp 293—294° (decomp.), $[\alpha]_D$+29° (c=2.6, EtOH) is crystallized from aqueous methanol and shows the infrared (IR) absorptions at ν_{max}^{KBr} 3400, 1690, and 1630 cm^{-1}. The mass spectrum and elemental analysis indicate the formula $C_{30}H_{48}O_6$ (M^+ at m/e 504). The proton magnetic resonance (PMR) spectrum shows the absence of methoxyl and acetoxyl groups. Treatment of arjungenin with diazomethane gave a methyl ester (II), mp 162—165°, $C_{31}H_{50}O_6$ (M^+ at m/e 518.3503, Calcd. 518.3604) ν_{max}^{KBr} 3430, 1720, and 1640 cm^{-1}. Acetylation of the ester (II) with acetic anhydride and pyridine at room temperature gave an ester triacetate (III), mp 137—138°, $[\alpha]_D$+8.2° (c=1.82, EtOH), $C_{37}H_{56}O_9$ (M^+ at m/e 644), ν_{max}^{Nujol} 3520, 1740, and 1630 cm^{-1}, PMR (Table I), which still shows an IR absorption due to a hydroxyl group. When treated with acetic anhydride in the presence of perchloric acid, III gave an ester

1) *a*) L.R. Row, P.S. Murty, G.S.R.S. Rao, C.S.P. Sastry, and K.V.J. Rao, *Indian J. Chem.*, **8**, 716 (1970); *b*) L.R. Row, P.S. Murty, G.S.R.S. Rao, C.S.P. Sastry, and K.V.J. Rao, *Indian J. Chem.*, **8**, 772 (1970); *c*) L.R. Row and G.S.R.S. Rao, *J. Indian Chem. Soc.*, **39**, 89 (1962).
2) F.E. King, T.J. King, and J.M. Ross, *J. Chem. Soc.*, **1954**, 3995.