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Summary

A new nucleoside, 3-(β-D-ribofuranosyl)-2-oxo-2,3-dihydropyrimidine (6-deoxyuridine), was synthesized from 2-hydroxypyrimidin-1-ylmercury chloride and 2,3,5-tri-O-benzoylribofuranosyl chloride, followed by removal of protecting groups. 6-Deoxyuridine 5′-monophosphate and 2′(or 3′), 5′-diphosphate were obtained by phosphorylation of 6-deoxyuridine.

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57. Hiroshi Mitsuhashi and Yuzuru Shimizu: Studies on the Constituents of Asclepiadaceae Plants. I.[1] On the Components of *Cynanchum caudatum* MAX.

(*Faculty of Pharmacy, Medical School, University of Hokkaido*[*1])

Cynanchum caudatum MAX. (Japanese name, Ikema. Asclepiadacea family) is a plant widely distributed in Japan especially in the Hokkaido and its root has been used as a crude drug among the people of the Ainu race, and the natives of Hokkaido and Sakhalin have employed it for all kinds of diseases as a home remedy[2] (Ainu name: Ikema or Penup). This plant, however, is very toxic and sometimes causes very serious poisoning with vomiting, diarrhea, convulsion, arhythmea, etc.[3]

Concerning the components of the root of this plant, Kunitomo[4] reported the isolation of sucrose and an alkaloidal substance, about which not much detail was given. While Iwakawa gave a name cynanchotoxin[*2] to the ether-soluble and petroleum ether-insoluble fraction of the ethanol extract, and reported that it had picrotoxin-like action and LSD 0.002 g./20 g. frog,[5] it now seems that the substance was a crude mixture. Further investigation was attempted in order to determine the components of the root of this plant.

Percolation of the powdered root with chloroform afforded a powdery extract, which showed strong Keller-Kiliani reaction (blue), suggesting the presence of a glycoside containing 2-deoxy-suger component. Active methylene reaction was negative in the extract. Therefore, it is reasonable to assume that the glycoside is not a cardiac glycoside. The extract was precipitated several times with petroleum ether and the crude glycoside

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*1 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (三橋 博, 清水 譲).

*2 N. Nagai reported the isolation of phytolaccotoxin from the root of *Phytolacca esculenta* VAN HOUTTE (Yakugaku Zasshi, **10**, 214(1890)), but according to Iwakawa, phytolaccotoxin should be cynanchotoxin itself, and this may have been caused by confusion of the starting material, owing to the resemblance of the appearance of these roots.

1) Part of this work was reported at the 3rd Hokkaido Local Meeting of the Pharmaceutical Society of Japan, July 27, 1959.
2) M. Chiri: "The Dictionary of Ainu Language," Vol. I (1953). Oka Shoin.
3) Y. Narumi: Tohoku J. Med., **19**, 439(1936).
4) Y. Kunitomo: Yakugaku Zasshi, **18**, 653(1898).
5) K. Iwakawa: Tokyo J. Med., **26**, 359(1912); Arch. exptl. Path. Pharmakol., **67**, 118(1912).

thus gained was chromatographed over an alumina column. Each fraction was white or faintly colored yellow, positive to the Keller–Kiliani reaction, and an amorphous powder, and their infrared spectra resembled each other. From this fact, it is considered that the glycosides are a very closely related glycoside mixture and that it is rather difficult to separate them into pure substances. The crude glycoside was hydrolysed with 0.05*N* sulfuric acid in 50% methanol, the same condition usually applied to the hydrolysis of cardiac glycosides containing 2-deoxy-sugar,[6] and after removal of methanol, extraction with ether gave a Keller–Kiliani-negative aglycone mixture. The mixture was chromatographed over an alumina column to give two fractions (see Table III), one of which crystallized from ether–petroleum ether as fine long needles, m.p. 167°, and showed the Lieberman–Burchard reaction of pink→yellow, and the other, an amorphous white powder, gave orange→green coloration. The former was named cynanchogenin*3 but the latter seems to be impure and infrared spectral studies showed it to be very similar to the former.

For preparative purposes, the direct hydrolysis of chloroform extract was carried out, using a smaller amount of 0.05*N* sulfuric acid in 50% methanol than described above. From 450 g. of the extract, 180 g. of crude aglycone mixture was obtained. Chromatography of the mixture through alumina yielded three crystalline substances. One of them was cynanchogenin and one of the remaining two showed m.p. 218° after numerous recrystallization from acetone. The infrared spectrum exhibited ester (acetate) bands at 1240 and 1720 cm^{-1}, terminal methylene at 900 and 1650 cm^{-1}, and characteristic absorptions at 1026, 1015, and 980 cm^{-1} suggested a triterpene having equatorial acetyl.[7] Considering these facts, this substance seemed to be lupeol acetate and this was confirmed from elemental analyses. The other, m.p. 137~138°, showed a positive Lieberman–Burchard reaction and the mixed melting point of the acetate of this compound, m.p. 127~128°, with that of authentic β-sitosterol[8] gave no depression. The infrared spectrum was also the same as that of authentic sample. Judging from the results of chromatography of the glycoside and its hydrolysate, these two substances must be present as free forms in the plant.

The sugar part which had been obtained by hydrolysis of the glycoside was examined. The aqueous layer, after extraction of aglycone, displayed a strong Keller–Kiliani reaction (violet→bule→green). The paper partition chromatographic studies[9,10,11] of both sugar syrups from glycoside and chloroform extract showed only one spot at the Rf value similar to that of D-cymarose. The extract of the spot on the paper presented a positive Keller–Kiliani reaction. It is therefore doubtless that the spot is 2-deoxy-sugar. The extraction of sugar syrup with ether followed by high vacuum distillation gave a colorless syrup, which was oxidized with bromine water and distilled again to syrupy lactone which gave with phenylhydrazine, cymaronic acid phenylhydrazide, m.p. 152~154°.[12]

D-Cymarose is the sugar which mainly occurs in cardiac glycosides. Among a few exceptions, the cases of interest are drevoside-A from *Dreagea volubilis*,[10] condurangin from *Marsdenia Condurango*,[11] and vincetoxin from *Cynanchum vincetoxicum*,[11] which

*3 Details of cynanchogenin are to be reported in the following paper.

6) R. E. Winkler, T. Reichstein : Helv. Chim. Acta, **37**, 737(1954).
7) L. L. Allsop : J. Chem. Soc., **1956**, 4869.
8) H. Mitsuhashi, *et al.* : Yakugaku Zasshi, **78**, 539(1958).
9) S. Partridge, *et al.* : Biochem. J., **42**, 241(1949).
10) T. Reichstein : Helv. Chim. Acta, **37**, 743(1954).
11) F. Korte : Chem. Ber., 88, 1533(1955).
12) R. C. Elderfield : J. Biochem., **111**, 527(1935).

all contain D-cymarose as the sugar component, and these plants belong to Asclepiadaceae family. Here exist a series of non-cardiac glycosides containing 2-deoxy-sugar in Asclepiadaceae family and the fact is of taxonomical and biogenetic interest.

Experimental

Extraction Test—The root of *Cynanchum caudatum* MAX., gathered at Nishino, a suburb of Sapporo city, on June 4, 1958, was chipped, dried, and powdered. The powdered root was extracted with various solvents and the residues from evaporation of the solvents were weighed.

TABLE I. Extraction with Various Solvents and Color Test of the Extracts

Expt. No.	Solvent	Yield of extract (%)	Keller-Kiliani reaction	Lieberman-Burchard reaction	Raymond and Legal test
1	Hexane	3.3	green	red→green	—
2	Et_2O	7.2	blue	〃	—
3	$CHCl_3$	12.7	〃	〃	—
4	AcOEt	10.4	blue→green	〃	—
5	Me_2CO	26.1	blue	〃	—
6	EtOH	28.9	〃	〃	—
7	Et_2O (after extn. with hexane)	4.3	〃	pink→yellow-green	—
8	$CHCl_3$ (after extn. with Et_2O)	3.5	〃	red→green	—
9	EtOH (after extn. with $CHCl_3$)	21.1	—	yellow-brown	—

20 g. of each sample was extracted in a Soxhlet apparatus for 24 hr.
Keller-Kiliani reaction according to the method of T. Reichstein (Helv. Chim. Acta, **31**, 888(1948)).
From Nos. 5, 6, and 9 a good amount of sucrose was obtained.

Experiment with Chloroform Extract—One kg. of ground dry root was percolated with $CHCl_3$ at room temperature and 45 g. of a faintly yellow-colored amorphous powder was obtained. The powder was reprecipitated several times with hexane to remove oily substances. Three g. of the resulting crude glycoside was chromatographed over 100 g. of neutral alumina and the results are shown in Table II. Several efforts to crystallize the eluted product were in vain.

TABLE II. Column Chromatographic Analysis of the Crude Glycoside

Fract. No.	Solvent	Eluted product (mg.)	Keller-Kiliani reaction
1～5	$CHCl_3$	trace	violet→blue
6～7	MeOH-$CHCl_3$ (1:99)	111	〃
8～13	(3:97)	239	〃
14～22	(5:95)	28	〃
23～28	(10:90)	12	〃
29～32	(20:80)	trace	〃
33～40	(50:50)	289	〃
41～46	(70:30)	370	〃
47～53	MeOH	286	〃
54～62	MeOH-H_2O (90:10)	1,048	〃
63～64	(50:50)	150	〃

Each fraction : 50 cc.

Hydrolysis of the Glycoside—Two g. of the crude glycoside (precipitated with hexane) was dissolved in 60 cc. of MeOH, after addition of 60 cc. of 0.1*N* H_2SO_4, and refluxed for 25 min. MeOH was evaporated *in vacuo* at room temperature and the residue was extracted with Et_2O. The ether layer was washed with 5% $NaHCO_3$ solution and water, and dried over Na_2SO_4. Removal of the solvent gave 550 mg. of a powder (Keller-Kiliani reaction, negative), which was chromatographed over 25 g. of alumina (neutral, activated at 200°).

Direct Hydrolysis of the Chloroform Extract—$CHCl_3$ extract was dissolved in MeOH and the insoluble part was centrifuged off. To 10 g. of the MeOH-soluble part, 40 cc. of MeOH and 20 cc. of 0.15*N* H_2SO_4 were added and the mixture was refluxed for 30 min. MeOH was evaporated *in vacuo* at room temperature and the resulting mixture was extracted with Et_2O. The ether layer, treated

Table III. Chromatography of the Hydrolysate

Fract. No.	Solvent (cc.)	Eluted product (mg.)	Lieberman-Burchard reaction
1	Benzene 100	—	—
2	5% $CHCl_3$-Benzene 100	—	—
3	20% $CHCl_3$-Benzene 100	—	—
4	50% $CHCl_3$-Benzene 100	—	—
5	$CHCl_3$ 100	—	—
6	1% MeOH-$CHCl_3$ 100	242	pink→yellow
7	5% MeOH-$CHCl_3$ 100	—	—
8	5% MeOH-$CHCl_3$ 100	195	orange→green
9	5% MeOH-$CHCl_3$ 100		

as usual, afforded 4.6 g. of a residue after removal of Et_2O. The residue showed practically a negative Keller-Kiliani reaction.

Chromatography of the Hydrolysate of the Chloroform Extract—Eighty g. of the residue was submitted to chromatography over 1.6 kg. of alumina (neutral), giving results shown in Table IV.

Table IV. Chromatography of the Hydrolysis Product of the Chloroform Extract

Fract. No.	Solvent	Eluted product (g.)	Note	Fract. No.	Solvent	Eluted product (g.)	Note
1~3	$CHCl_3$		—	22~24	MeOH-$CHCl_3$ 1:99	4.0	crystal+amorphous substance
4	〃	4.0	oil+crystal (1)	25~26	〃	20.0	
5	〃	4.9	oil	27~28	〃	12.0	
6	〃	2.4	oil	29~30	〃	4.0	Lieberman-Burchard pink→brown
7	〃	1.1	oil+crystal (2)	31~32	〃	1.0	
8	〃	1.2		33~34	〃	0.5	
9	〃	1.4		35~45	MeOH-$CHCl_3$ 50:50	15.0	amorphous Lieberman-Burchard green
10~14	〃	2.2					
15~21	〃	2.5					

Each fraction : 500 cc.

The fraction Nos. 22~34 gave cynanchogenin, m.p. 167°, after repeated recrystallization from Et_2O-petr. ether.

Crystal No. 1 (Lupeol Acetate)—The oily fraction Nos. 1~3 gave a crystalline mass on standing for a few days. This crystalline mass was recrystallized from Me_2CO many times to give needles, m.p. 216°. Lieberman-Burchard reaction, reddish-violet; tetranitromethane test, strongly positive. IR λ_{max}^{Nujol} cm^{-1} : 1720, 1240, 1026, 1015, 998, 980, 964, 900. *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found : C, 82.20, 81.86; H, 10.95, 10.89.

Crystal No. 2 (β-Sitosterol)—Fraction Nos. 7~14 was recrystallized from petr. ether to long needles, m.p. 137~138°. Lieberman-Burchard reaction showed color change of pink→blue→green. The mixed m.p. with authentic β-sitosterol (m.p. 137~138°) melted at 137~138°. *Anal.* Calcd. for $C_{29}H_{50}O$: C, 83,99; H, 12,55. Found : C, 84,31; H, 11.98. IR λ_{max}^{KBr} cm^{-1} : 3420, 1650, 1060, 805.

The acetate was prepared by treating with Ac_2O in pyridine. Leaflets (from MeOH), m.p. 127~128°. The mixed m.p. with authentic sample (m.p. 127~128°) showed no depression. *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.51; H, 11.48. Found : C, 81.97; H, 10.92.

Paper Chromatography of the Sugar—The aqueous layer, obtained on hydrolysis of the glycoside, and that from $CHCl_3$ extract were neutralized with freshly precipitated $BaCO_3$ and concentrated to a syrup under a reduced pressure. Paper chromatographic analysis was carried out on these two kinds of syrups, giving results shown in Table V.

Table V. Paper Partition Chromatography of the Sugar Portion

	Rf Value	
	(BuOH-1% NH_3)	(BuOH-pyridine-H_2O =3:1:3)
Sugar from the glycoside (Fract. No. 29)	0.70~0.73	0.73~0.76
Sugar from $CHCl_3$ extract	0.70~0.73	0.73~0.76
D-Cymarose	0.73	0.73~0.76

Toyo Roshi No. 51, descending method, detected by 0.1*N* $AgNO_3$ and 5.0*N* NH_3 (by Partridge)

The appropriate area of the spot was extracted with acetone and tested by the Keller-Kiliani reaction to give positive result.

Cymaronic Acid Phenylhydrazide from the Sugar Syrup—The sugar syrup from $CHCl_3$ extract was extracted with Et_2O. Almost all was soluble in Et_2O and a small amount of insoluble matter gave no spot on the paper chromatogram. 500 mg. of the ether-soluble sugar syrup was distilled at 0.007 mm. Hg at 110° (bath temp.), affording 400 mg. of colorless distillate giving strongly positive Keller-Kiliani reaction.

To a solution of 200 mg. of the distillate in 5.6 cc. of water, 0.132 cc. of Br_2 was added and the mixture was allowed to stand for 23 hr. in the dark at 20°. After evaporation of excess Br_2 under a reduced pressure, the reaction mixture was neutralized to pH 6.0 with freshly precipitated Ag_2CO_3 and filtered. The filtrate was treated with H_2S at 0° and Ag_2S was filtered off. Evaporation of the filtrate under a reduced pressure afforded a syrupy residue, which was distilled at 0.005 mm. Hg at 130° (bath temp.). The distillate was heated with phenylhydrazine at 100° for 40 min. The reaction product gave needles, m.p. 144~151°, from Et_2O. Repeated crystallization from Et_2O-MeOH afforded long needles, m.p. 152~154° (not analyzed).

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Summary

The root of *Cynanchum caudatum* MAX. was proved to contain a glycoside mixture, β-sitosterol and lupeol acetate. The glycosides showed a strong Keller-Killiani reaction, suggesting the presence of 2-deoxy-sugar. The sugar portion of the glycosides was found to be D-cymarose by paper partition chromatography comparing with an authentic specimen and by preparation of cymaronic acid phenylhydrazide, m.p. 152~154°. One of the aglycone, cynanchogenin, m.p. 167°, was isolated by chromatography using alumina.

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