Communications to the Editor

Presence of Digitalinum verum and Purpurea Glycoside B in the Water-soluble Fraction of the Dried Leaves of *Digitalis purpurea* L. (Addendum).

Digitalinum verum has hitherto been isolated only from the seeds of *Digitalis purpurea* and *Digitalis lanata*. Mohs[1] once suggested the presence of monoacetyl derivative of the glycoside in the leaves of *Digitalis lanata*. Most recently Tschesche[2], *et al.*, demonstrated in the leaves the existence of 16-acetyl-digitalinum verum, which was only separated as the hexaacetate from the unseparable mixture with a new glycoside "Gitorin".

For years, we have been seeking water-soluble cardioactive principles from the digitalis leaves other than the known glycosides, since it was believed from the pharmacological points of view, that the water-soluble extract of the dried leaves should contain a certain less cumulative but rather active ingredient.

Some years ago, one of the writers (Ishidate) and Takemoto[3] reported a new type of glycoside which they designated as "digicorin", and was tentatively assumed as an acidic glycoside composed of acetylgitoxigenin and 2-desoxyhexuronic acid. However, subsequent experiments have not succeeded in confirming this fact as yet, and therefore, the name "digicorin" will be withdrawn from literature for the present.

This time, the present writers found that, in the water-soluble fraction of the leaves of *Digitalis purpurea*, a considerable amount of digitalinum verum is present besides purpurea glycoside B and a certain gitoxigenin-glycoside of high toxicity. These three components should play an important part in the pharmacological activities of the leaves.

The leaves of *D. purpurea*, cultivated in the Medicinal Plant Station of Takeda Pharmaceutical Industries, Ltd., were dried at 60° in an electric oven with a blower, and extracted with methanol. The methanol extract was digested with water, or better with bicarbonate solution, the aqueous solution was washed with ethyl acetate, and then extracted with chloroform-alcohol (2 : 1) mixture. The chloroform-alcohol extract was purified with ether and lead acetate. The crude water-soluble "tannoids" were separated by partition chromatography employing a column of active charcoal, into the following four fractions.

Fr. I (Methanol fraction). This gave negative Legal and Keller-Kiliani reactions. Succinic, citric, and caffeic acids were identified from this fraction.

Fr. II (Chloroform-alcohol (2 : 1) fraction). This fraction gave a positive Legal test and, by the Keller-Kiliani reaction, no coloration in upper layer while a distinct red shade (characteristic to gitoxigenin) at the zone of contact.

Fr. III (Chloroform-alcohol (2 : 1) fraction). This gave a positive Legal test and gave, by the Keller-Kiliani reaction, a bluish green color (digitoxose !) in the acetic acid layer and a red shade in the sulfuric acid layer.

Fr. IV (Chloroform-alcohol (2 : 1) fraction). This fraction gave no Legal reaction and, by Keller-Kiliani reaction, no coloration in the upper layer while in the sulfuric acid layer a slight brown shade was noticed.

Of these fractions, Frs. II and III are the principal components of the tannoids and pharmacologically significant.

Presence of digitalinum verum and a certain gitoxigenin-glycoside.—Fr. II formed a colorless powder after purification with lead hydroxide. It was separated into two parts, one of acetone-insoluble and the other of soluble portions. The former (Fr. IIA), after recrystallisation from methanol-ether yielded a pseudo-crystalline powder of m.p. 228~230° (decomp.) (uncorr.), $[\alpha]_D^{21}$:+5.7° (CH_3OH). The yield was estimated as 0.02~0.03% of the dried leaves. It was insoluble in chloroform, acetone, or ether, while soluble in ca. 500 parts of water. It gave a strong positive Legal test and by the Keller-Kiliani reaction, no coloration in acetic acid layer but a red shade at the zone of contact. It possessed no acetyl group, and the analytical and physical

1) P. Mohs: Arch. Pharm., 283, 93 (1950).
2) R. Tschesche, G. Grimmer, F. Neuwald: Ber., 85, 1103 (1952).
3) M. Ishidate, T. Takemoto: Acta Phytochim., 15, 201 (1948).

data were very similiar to those of digitalinum verum. By hydrolysis with alcoholic hydrochloric acid (3.4%) of the substance, an aglycone of m.p. 208~210° (from methanol) was obtained which was proved to be indentical with dianhydrogitoxigenin by the mixed fusion test as well as the absorption spectrum. As the sugar components, the presence of glucose and digitalose was verified by the Rf values (0.200 and 0.530, respectively) of the paper chromatogram*.

The Substance IIA (dried at 110° for 7 hrs. in vacuo).

Anal. Calcd. for $C_{36}H_{56}O_{14}$ (Digitalinum verum): C, 60.66; H, 7.92; OCH_3, 4.36. Found: C, 60.58; H, 8.14; OCH_3, 3.69, 3.47, 2.55.

The mixed fusion test with digitalinum verum, m.p. 233° (decomp) (uncorr.), isolated from the seeds of *D. purpurea*, showed no depression. The hexaacetyl derivative of the substance (m.p. 172~175°/220~225°) also coincided with that of the authentic preparation.

Anal. Calcd. for $C_{48}H_{68}O_{20}$ (Digitalinum verum hexaacetate): C, 59.73; H, 7.12; CH_3O, 3.25. Found: C, 59.90; H, 7.41; CH_3O, 3.36. $[\alpha]_D^{18}$: $-14.5° \pm 0.9°$ (c=0.5536, in $CHCl_3$).

However, the yield of the hexaacetate was comparatively low and variable according to the material substances as was also the case of the methoxyl content of the substance IIA. Moreover, it was seen that the substance IIA represented a far higher toxicity and a different cumulative character in the animal test from those of digitalinum verum (see Table I).

The repeated recrystallisation as well as partition chromatography on alumina or on silica gel have proved to be of little value in further purification or separation.

From the acetylation product of the substance IIA, digitalinum verum hexaacetate was easily isolated as the benzene-insoluble portion, while a considerable amount of amorphous powder was obtained from the benzene-soluble fraction. Further studies on the latter are under way.

Therefore, it can reasonably be assumed that the substance IIA is not a single compound but a mixture of digitalinum verum and an unknown glycoside (presumably having gitoxigenin as the aglycone) of high toxicity, in a ratio of about 6:4 to 7:3 in a practically inseparable state.

The acetone-soluble fraction, IIB, when subjected to alumina-chromatography, yielded a white amorphous powder. It gave a positive Legal and digitoxose reactions and one spot (Rf:0.07), corresponding to that of purpurea glycoside B, by paper chromatography. By a mild hydrolysis it was converted into gitoxigenin, and digitoxose and digilanidobiose, all of which were identified through comparison with authentic preparations by the paper chromatographic procedure.

Isolation of purpurea glycoside B—Fr. III, the second major component of the tannoids, was subjected to alumina chromatography using chloroform-alcohol as developer for further purification. The colorless powder obtained after being washed with water and carbonate solution was recrystallised from ethanol, to white crystalline powder having m.p. of 233~234° (uncorr.). The analytical data as well as chemical nature proved to be identical with those of purpurea glycoside B of Stoll and Kreis[4]. The total yields obtained from Fr. III and Fr. IIB were estimated as ca. 0.03% of the dried leaves.

Anal. Calcd. for $C_{47}H_{74}O_{19}$ (Purpurea glycoside B) (70° in vacuo, 5 hrs.): C, 59.83; H, 7.91. Found: C, 59.82; H, 7.77. $[\alpha]_D^{20}$: $+19.6 \pm 1.0$ (c=0.485, 75% CH_3OH).

TABLE I Comparison of Toxicities of Glycosides**

	Frog—Min. fatal dose (4 hrs.), mg. per g.	Cat—Fatal dose (H.M. method) mg. per kg.	Pigeon—Cumulative effect (%) 3 hrs.	6 hrs.	24 hrs.
Fr. IIA No. 1	0.0008	0.540	42.86	0	
No. 2	0.00095	0.655	25.46	0	
Digitalinum verum (from seeds)	0.0012	0.911		34.06	0
Purpurea glycoside B (from Fr. III)	0.0006	0.460		23.4	2.3
Digitoxin	0.0011	0.420			89.68 60.50 (48 hrs.)

**These experiments were carried out by Dr. K. Tokita, Dr. Y. Aramaki and Mr. K. Kikuchi at the Pharmacological Institute, University of Tokyo. The details will be reported elsewhere. The data represent the average value of 5 or 6 cases.

* In the paper chromatography, solvent used was butanol-pyridine-water (5:3:1) and filter paper, Toyo Roshi No. 131.

4) A. Stoll, W. Kreis: Helv. Chim. Acta, 18, 120 (1935).

It is noted that the above three components all belong to glycosides of gitoxigenin, and glycosides of digitoxigenin series were found only in a negligible amount in the leaves collected so far at the above-mentioned plant garden.

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A Synthesis of rac-C-trisnoremetine.

An abstract of the paper by Pailer, *et al.*, upon the synthesis of rac-C-noremetines appeared in the recent number of Chemical Abstracts (47, 2186). Our synthesis of rac-C-trisnoremetine, which has been completed nearly a year ago and has to be published jointly with other syntheses in the related field, now under progress in our hands, covered almost the same ground with theirs. But certain discrepancies in properties of some of the compounds cited were observed.

N-β-3,4-Dimethoxyphenethyl-4-carbethoxypyridinium bromide, m.p. 196°, was oxidized by means of alkaline potassium ferricyanide solution, yielding the corresponding pyridone carboxylic acid, m.p. 233~234° (decomp.), in a good yield. This was then ring-closed with phosphoryl chloride and the resultant compound was treated with absolute alcohol, giving rise to 4',5'-dimethoxy-7-carbethoxy-3,4,5,6,7,8-hexahydro-9,10-dehydro-(2',1' : 1,2-benzoquinolizinium) salt, m.p. 182~184° (decomp.) (as iodide), which, on being reduced catalytically, furnished the corresponding oily tertiary base. The latter forms crystalline hydrazide, m.p. 204~207°, the azide of which was treated with homoveratrylamine in ethereal solution, giving the corresponding amide, 4',5'-dimethoxy-3,4,5,6,7,8-hexahydro-(2',1' : 1,2-benzoquinolizyl)-7-carboxylic acid homoveratramide of m.p. 154~157° in fair yield. This was then ring-closed and reduced, yielding rac-C-trisnoremetine as colorless crystalline solid with melting range of 97~107° (decomp.), which is fairly unstable in the air, turning gradually red. The dipicrate and dihydrochloride, both having indefinite melting points, were also prepared.

The detail will be published in the forthcoming number of this Bulletin.

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