above bromophenanthrenecarboxylic acid (XIV), 15 cc. of *N* NaOH, 5 cc. of EtOH, and 2 g. of copper bronze was refluxed for 39 hr. After cool, the mixture was filtered and the filtrate was acidified with conc. HCl. The precipitate was recrystallized from EtOH to almost colorless prisms, m.p. 294~296°(decomp.), which showed no depression when mixed with a specimen prepared from (V). Yield, 0.3 g. *Anal.* Calcd. for $C_{17}H_{10}O_6$: C, 65.81; H, 3.25. Found : C, 65.60; H, 3.51.

α-(3,4-Methylenedioxyphenyl)-3,4-methylenedioxycinnamic Acid (VII)——This compound was prepared from 1.5 g. of piperonal, 2 g. of sodium homopiperonylate, and 10 cc. of Ac_2O in the same manner as for (XII). The crude product was recrystallized from EtOH to colorless needles, m.p. 243~244°. Yield, 1.2 g. *Anal.* Calcd. for $C_{17}H_{12}O_6$: C, 65.38; H, 3.87. Found: C, 65.08; H, 4.11. UV λ_{max}^{EtOH} mμ (log ε) : 294 (4.12), 324 (4.20).

On concentration of the EtOH mother liquor, a small amount of colorless needles, m.p. 233~234°, was obtained. The structure of this substance is still unknown.

Determination of Ultraviolet Spectra——The ultraviolet spectra were measured in EtOH solution using Hitachi EPU-2A spectrophotometer.

The authors wish to express their deep gratitude to Prof. M. Tomita of Kyoto University for his guidance. Thanks are due to Mr. K. Machida and Mrs. I. Hamanaka, Kyoto University, for the measurements of infrared spectra. The authors also wish to thank Mrs. M. Hasegawa and Mr. T. Ueda of this School for carrying out the microanalyses.

Summary

2,3:5,6-Bismethylenedioxyphenanthrene (X) and 2,3:6,7-bismethylenedioxyphenanthrene (XI) were synthesized from 6-nitropiperonal (I) and homopiperonylic acid (II) by the Pschorr reaction. The structure of the compounds was determined by an unequivocal synthesis using 6-bromopiperonylic acid. The ultraviolet absorption spectra of several kinds of phenanthrenes possessing methylenedioxy group at 2,3- or 3,4-position were determined in ethanolic solution.

(Received February 6, 1961)

UDC 615.711.5:547.918

7. Daisuke Satoh, Hiroshi Ishii, Yohko Oyama, and Tamotsu Okumura :
Studies on Digitalis Glycosides. XI.[*1] Isolation of Digipronin, Purpnin, and Purpronin.

(*Research Laboratory, Shionogi & Co., Ltd.*[*2])

From the leaves of Digitalis species, a number of noncardiac glycosides containing C_{21}-steroids as aglycones were isolated by several workers. These glycosides were known generally as the digitanol glycosides[1] and were classified into two types, the diginigenin type (basic formula I) and the pregnenolone type (basic formula II).[2] Diginin,[1,3] digi-

---

[*1] Part X : H. Ishii, Y. Nozaki, T. Okumura, D. Satoh : Yakugaku Zasshi, **81**, 805 (1961)

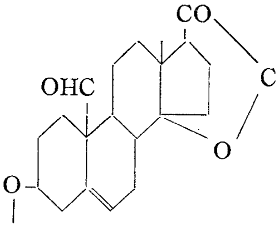
[*2] Fukushima-ku, Osaka (佐藤大助, 石井　宏, 尾山蓉子, 奥村　保).

1) R. Tschesche, G. Buschauer : Ann., **603**, 59 (1957).

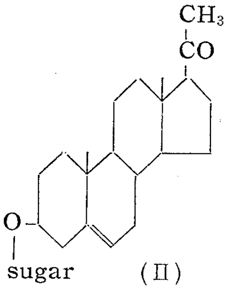
2) R. Tschesche : Proc. IVth International Congress Biochem., Vol. IV, 21 (1958).

3) W. Karrer : Festschrift für Barell, Basel, 236 (1936); C. W. Shoppee, T. Reichstein : Helv. Chim. Acta, **23**, 975 (1940); C. W. Shoppee : *Ibid.*, **27**, 246, 426 (1944); J. Press, T. Reichstein : *Ibid.*, **30**, 2127 (1947).

folein,[1,4~6] lanafolein,[1,4] and digitalonin[7] belong to the former type, and digipurpurin[5] and digacetinin[8] were presumed to belong to the latter type. In addition to these glycosides, several digitanol aglycones of an unknown type were also isolated.[9] As a new field of plant steroids, these digitanol glycosides have aroused much interest in connection with their biosynthesis and pharmacological activity.



(I)



(II)

In a series of commuications, the present authors reported briefly on the isolation of three new digitanol glycosides of the latter type, digipronin,[10,11],[*3] purpnin,[10,12] and purpronin,[12] from the leaves of *Digitalis purpurea* L. and on the results of studies on the structures of these glycosides. The properties and the results of hydrolysis of these glycosides and characterizations of their aglycones are described in this paper. A method of distinguishing the two types of digitanol glycosides is also reported. Details of the structures of aglycones of these new digitanol glycosides will be described in the forthcoming papers.

Crystal C, which was separated from fraction Nos. 31~37 of the secondary alumina chromatography (1)[7] of the water-soluble fraction of the leaves of *Digitalis purpurea* L., formed colorless needles, m.p. 235~238°, in approx. 0.0015% yield based on the dried leaves. This substance is soluble in methanol, ethanol, and chloroform, and slightly soluble in ether and benzene. UV: λ_{max}^{EtOH} 300 mμ (log ε 1.92), IR λ_{max}^{Nujol} μ: 5.77, 5.88. $[\alpha]_D^{28}$ −64.5° (c=1.051, pyridine). Toxicity by the pigeon method, 12 mg./kg. Though the Legal test was positive, the Raymond test was negative at room temperature but showed reddish orange color on heating in contrast with the glycosides of diginigenin type. From the data of toxicity, ultraviolet spectrum, and the Raymond test, crystal C seemed to be a non-cardiac glycoside. The Keller-Kiliani test, with colorless acetic acid layer and reddish brown contact zone, showed that the sugar moiety of crystal C was not a 2-deoxysugar. Analytical values corresponded to $C_{28}H_{40}O_9$, possessing one methoxyl group.

The above data showed that crystal C was a new glycoside hitherto not described in the literature. Therefore, this glycoside was designated digipronin.

Hydrolysis of digipronin by the Mannich method gave three aglycones and *d*-digitalose. The main aglycone formed colorless prisms, m.p. 239~244°; UV: λ_{max}^{EtOH} 296 mμ (log ε 1.90), IR λ_{max}^{KBr} μ: 2.75, 3.00, 5.76, 5.86, 7.33. $[\alpha]_D^{31}$ −87.5° (c=1.010, $CHCl_3$-MeOH (1:1)), which

---

[*3] R. Tschesche and his collaborators also isolated digipronin and published some results of studies on its structure. cf. R. Tschesche, G. Lipp, G. Grimmer: Ann., **606**, 160 (1957); R. Tschesche, G. Lipp: *Ibid.*, **615**, 210 (1958).

4) R. Tschesche, G. Lipp: Ann., **615**, 210 (1958).

5) R. Tschesche, G. Grimmer: Chem. Ber., **88**, 1569 (1955).

6) D. Satoh, K. Yoshida, H. Ishii, Y. Nishimura: This Bulletin, **1**, 396 (1953).

7) D. Satoh, H. Ishii, Y. Oyama, T. Wada, T. Okumura: *Ibid.*, **4**, 284 (1956); D. Satoh, T. Wada, H. Ishii, Y. Oyama, T. Okumura: *Ibid.*, **5**, 253 (1957).

8) R. Tschesche, W. Hammerschmidt, G. Grimmer: Ann., **614**, 136 (1958).

9) R. Repič, Ch. Tamm: Helv. Chim. Acta, **40**, 639 (1957).

10) D. Satoh, H. Ishii, Y. Oyama, T. Okumura: Yakugaku Zasshi, **75**, 1573 (1955).

11) D. Satoh: This Bulletin, **8**, 270 (1960).

12) D. Satoh, Y. Oyama, H. Ishii: *Ibid.*, **8**, 657 (1960).

showed a positive Liebermann and tetranitromethane test. Analytical values corresponded to $C_{21}H_{28}O_5$ and showed that this aglycone contained 3 $C-CH_3$ groups in its molecule analogous to the C_{21}-steroids of pregnenolone type. This main aglycone was designated α-digiprogenin. One of the two other aglycones forms pale yellowish brown needles, m.p. 190~192° and analytical values corresponded to $C_{21}H_{26}O_4$, which was named β-digiprogenin. The third aglycone, γ-digiprogenin, obtained from the chloroform extract of the aqueous mother liquor of the crude aglycones, formed colorless needles, $C_{21}H_{28}O_5$, m.p. 248~252°. The number of the combined digitalose molecule was shown to be one by its methoxyl content.

From the above results, digipronin seemed to be a new digitanol glycoside.

The two crude substances obtained from the fractions Nos. 8~24 and Nos. 41~47 of the secondary chromatography (2) described in the previous paper[7] were similar in respect to color reaction, solubility, and melting point, and therefore, the two substances were thought to be identical and named crystal D in the previous paper. From the results of later studies it was clarified, however, that the infrared spectra of two glycosides differed from each other and the two aglycones were not identical. Therefore, the substance obtained from the former fractions was called crystal E to distinguish it from crystal D isolated from the latter fractions.

Crystal D formed colorless fine needles, m.p. 282~287°, $[\alpha]^{20}_{D}$ −14.8°(c=1.420, $CHCl_3$-MeOH (1:1)). This substance is slightly soluble in methanol, ethanol, chloroform, and

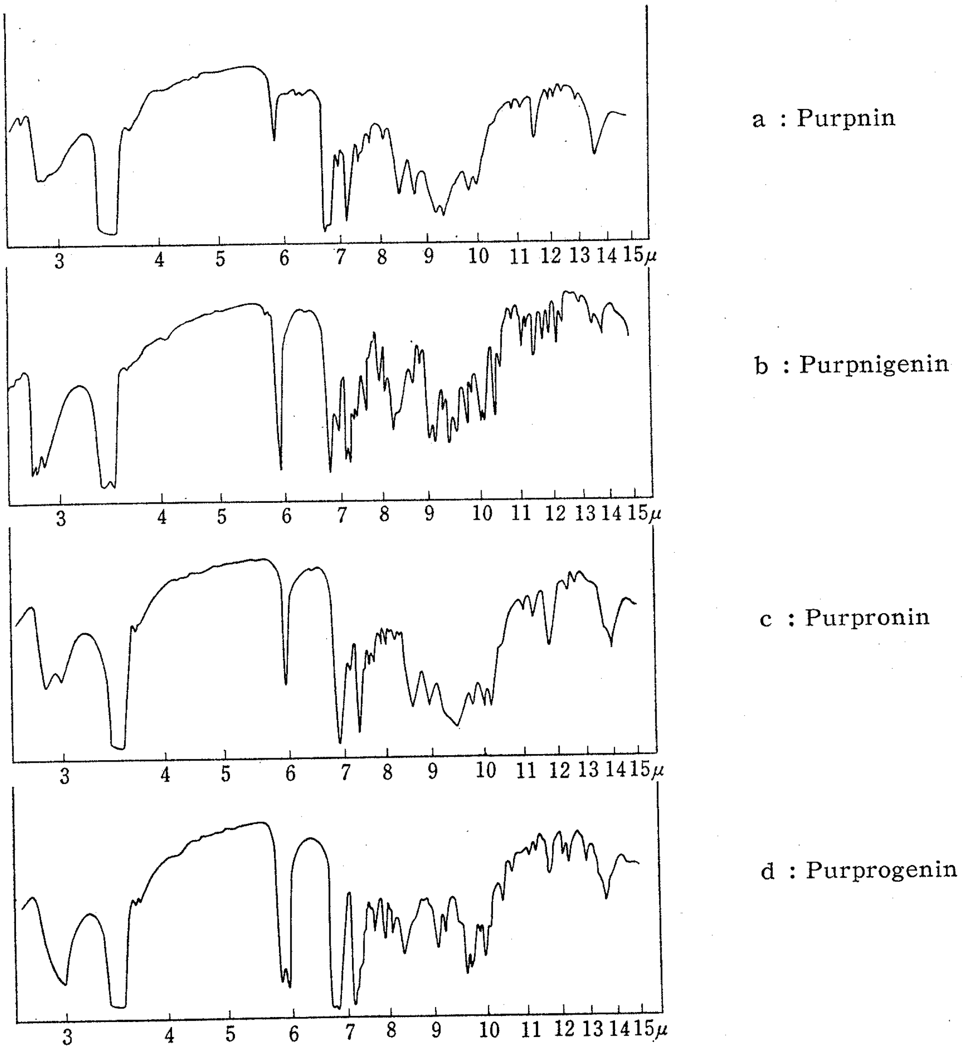


Fig. 1. Infrared Spectra

ethyl acetate, and soluble in a mixture of methanol or ethanol and chloroform. The infrared spectrum of crystal D showed a band of carbonyl group at 5.90 μ (Fig. 1a). UV: λ_{max}^{EtOH} 281 mμ (log ε 1.70). These data and failure of the Legal test indicated that this substance belongs to the non-cardiac glycoside. The Raymond test was negative at room temperature, but it exhibited a reddish-brown color on heating, analogous to digipronin. In the Keller-Kiliani test, a bluish-green acetic acid layer indicated that the sugar moiety of crystal D was a 2-deoxysugar. Analytical values were in good agreement with the formula $C_{39}H_{62}O_{13}\cdot H_2O$.

Hydrolysis of crystal D gave an aglycone as colorless plates, m.p. 239～243°, and *d*-digitoxose. This aglycone, UV: λ_{max}^{EtOH} 279 mμ (log ε 1.79); IR λ_{max}^{Nujol} μ: 2.81, 5.96, 7.32 (Fig. 1b); $[\alpha]_D^{16}$ +21.1° (c=1.140, MeOH), gave a positive Liebermann test and a tetranitromethane test. Analytical values corresponded to $C_{21}H_{32}O_4$ and showed that this aglycone contains 3 $C-CH_3$ groups in its molecule analogous to the C_{21}-steroids of pregnenolone type.

Calorimetric estimation using xanthydrol indicated the presence of three digitoxose in this glycoside. Based on these findings crystal D was assumed to be a new digitanol glycoside and this glycoside and its aglycone were therefore given the names of purpnin and purpnigenin, respectively.

Crystal E formed colorless fine crystals, m.p. 278～281°. This substance is slightly soluble in methanol, ethanol, chloroform, and ethyl acetate, and soluble in a mixture of methanol or ethanol and chloroform. The Legal and the Raymond tests were negative at room temperature, but the latter test gave a reddish-brown color on heating. These results indicated that crystal E belongs to the non-cardiac glycoside. The Keller-Kiliani test showed the presence of a 2-deoxysugar in the molecule of crystal E. Analytical values corresponded to $C_{39}H_{60}O_{14}$. The infrared spectrum of crystal E (Fig. 1c) showed a band of corbonyl group at 5.89 μ but the intensity of this band was stronger than that of crystal D at 5.90 μ. The finger-print regions of the infrared spectra of both substances resembled each other but that of crystal D lacked the band at 8.00 and 9.66 μ.

Hydrolysis of crystal E gave, along with *d*-digitoxose, an aglycone as colorless plates, m.p. 249～252°, which gave a positive Liebermann test. UV λ_{max}^{EtOH} 278 mμ (log ε 1.75); IR λ_{max}^{Nujol} μ: 2.98, 5.84, 5.92, 7.40 (Fig. 1d); $[\alpha]_D^{27}$ +60.0°±2° (c=1.06, MeOH). Analysis gave values corresponding to $C_{21}H_{30}O_5$. These data showed that the aglycone of crystal E differed from the aglycone of purpnin (purpnigenin) and possessed one more carbonyl group than the latter aglycone. Therefore, crystal E and its aglycone were designated purpronin and purprogenin, respectively. The number of molecules of digitoxose contained in purpronin was estimated as three by the similar calorimetric method as described above.

The results of color tests and ultraviolet absorptions of the cardiac glycosides and the digitanol glycosides isolated in the present series of work are listed in Table I. These characteristics are useful for discrimination between cardiac and digitanol glycosides or between two types of the latter glycosides.

TABLE I.

Digitanol glycosides	Legal test	Raymond test		UV λ_{max} (mμ)
		at room temp.	on heating	
Diginigenin type (diginin, digifolein, digitalonin)	±	violet	dark violet	～310
Unknown type* (digipronin, purpnin, purpronin)	−～±	——	reddish brown	280～300
Cardiac glycosides	++	blue	dark blue	～218

* This type was clarified as pregnenolone type from the results of later studies as will be described in forthcoming papers.

Experimental

1) Crystal C (Digipronin)

i) Isolation of Crystal C——The fraction Nos. 31∼37 obtained by the secondary chromatography (1) over alumina of the water-soluble fraction of the leaves of *Digitalis purpurea* L., reported in a previous paper,[7] was treated with AcOEt and afforded crude crystals which were recrystallized from MeOH to crystal as colorless needles, m.p. 235∼238°. On recrystallization of the fractions Nos. 12∼13 of the primary alumina chromatography from MeOH, crystal C was also obtained as a less-soluble constituent while crystal B (strospeside) remained dissolved in the mother liquor. The properties of crystal C are given in the main text. *Anal.* Calcd. for $C_{28}H_{40}O_9$: C, 64.59; H, 7.74; OCH_3, 5.96. Found : C, 64.75; H, 7.75; OCH_3, 6.24.

ii) Hydrolysis of Crystal C by the Mannich Method——Suspension of 3 g. of crystal C in 300 cc. of Me_2CO containing 3 cc. of conc. HCl was allowed to stand for 6 days at room temperature. Crystal C dissolved completely after 4 days. The solution was diluted with 200 cc. of water, neutralized with 5% NaOH, and concentrated in a reduced pressure. Crude aglycone (1.57 g.) which separated was collected on a filter and recrystallized from MeOH to α-digiprogenin as colorless prisms, m.p. 239∼244°, yield, 1.09 g. The properties of this aglycone are described in the main text. *Anal.* Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found : C, 70.08; H, 7.87.

Concentration of the mother liquor from α-digiprogenin gave β-digiprogenin as pale yellowish brown needles, m.p. 190∼192°, yield, 0.2 g. *Anal.* Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.45; H, 7.86.

The results of C-CH_3 determinations of α- and β-digiprogenin and some C_{21}-steroids by the Kuhn-Roth method were as follows :

α-digiprogenin 7.93%; β-digiprogenin 8.17%
progesterone 9.71%; 17α-hydroxypregnenolone 7.78%
16-dehydropregnenolone 8.23%; Reichstein's compound S 4.31%

Extraction of the aqueous filtrate from the crude aglycone with $CHCl_3$ and concentration of the $CHCl_3$ gave, on crystallization from AcOEt, γ-digiprogenin as colorless needles, m.p. 248∼252°, which depressed the m.p. of α-digiprogenin; yield, 0.18 g. *Anal.* Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found : C, 70.41; H, 7.94.

The aqueous layer mentioned above was concentrated in a reduced pressure and the syrupy residue was treated with dry Me_2CO to remove NaCl. The Me_2CO extract was concentrated and dried in a vacuum to give the sugar moiety of digipronin as a colorless syrup; yield, 0.63 g. This sugar was compared with *d*-digitalose on paper chromatography and the results were as follows :

Rf (BuOH-AcOH-H_2O=4:1:1) : Sugar of crystal C 0.53
d-Digitalose 0.53

2) Crystal D (Purpnin)

i) Isolation of Crystal D——The fractions Nos. 41∼47 of the secondary chromatography (2) were triturated with AcOEt and the pale yellowish brown powder thus obtained was recrystallized from a mixture of MeOH and $CHCl_3$ (1:1) to crystal D as colorless fine crystals, m.p. 282∼287°. The properties of this substance are given in the main text. *Anal.* Calcd. for $C_{39}H_{62}O_{13}\cdot H_2O$: C, 61.86; H, 8.52. Found : C, 62.17; H, 8.49.

ii) Hydrolysis of Crystal D——A solution of 500 mg. of crystal D in 50 cc. of 0.35% HCl (50% EtOH) was refluxed for 30 min. on a water bath and the solution was neutralized and concentrated in a reduced pressure. The aglycone which deposited was recrystallized from AcOEt to purpnigenin as colorless plates, m.p. 239∼243°. The properties of this aglycone are described in the main text. *Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26; mol. wt., 348.5. Found : C, 72.26; H, 9.35; mol. wt. (Rast), 380.0. C-CH_3, 7.73; pregnenolone (control), 7.80.

From the $CHCl_3$ extract of the aqueous filtrate, a small amount of purpnigenin was obtained.

The aqueous layer was concentrated in a reduced pressure, the syrupy residue was treated with dry Me_2CO to remove NaCl, and a sugar was obtained as a colorless syrup after evaporation of Me_2CO. Rf value of this sugar was compared with that of *d*-digitoxose and gave the following result.

Rf (BuOH-AcOH-H_2O=4:1:1) : Sugar of crystal D 0.65
d-Digitoxose 0.65

iii) Determination of Content of Digitoxose combined in Crystal D Molecule——1.495 mg. of digitoxin (containing 3 mole of digitoxose in its molecule) was dissolved in 100 cc. of a mixture of MeOH and $CHCl_3$ (1:1), and 2 cc. of this solution was evaporated. The residue (29.90 γ of digitoxin which contains 15.26 γ of digitoxose), mixed with 5 cc. of xanthydrol reagent (10 mg. of xanthydrol in 100 cc. of AcOH containing 1 cc. of conc. HCl), was heated in a boiling water-bath for 3 min., cooled in ice-water for 5 min., allowed to stand for 20 min. at room temperature, and the optical density at 540 $m\mu$ was determined. Crystal D was treated by the similar procedure and the content of digitoxose was calculated from the ratio of absorbancy of the two glycosides.

Sample (γ)		Digitoxose (γ)	Optical density	Content of digitoxose	
				Calcd. for 3 mol.	Found
Digitoxin (used as standard)	29.90	15.26	0.401	51.04	
Crystal D	30.66	15.72	0.413	51.58	51.27

3) **Crystal E (Purpronin)**

i) **Isolation of Crystal E**——The fraction Nos. 8~24 of the secondary chromatography (2) was triturated with AcOEt to give a pale yellowish brown powder which on recrystallization from a mixture of MeOH and $CHCl_3$ yielded purpronin as colorless fine crystals, m.p. 278~281°. The properties of this substance are described in the main text. *Anal.* Calcd. for $C_{39}H_{60}O_{14}$: C, 62.21; H, 8.03. Found : C, 61.95; H, 7.92.

ii) **Hydrolysis of Crystal E**——700 mg. of purpronin was hydrolyzed by the method similar to that for crystal D and the crude aglycone was recrystallized from dil. MeOH to purprogenin as colorless plates, m.p. 249~252°. The properties of this aglycone are described in the main text. *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found : C, 69.89; H, 8.30.

From the $CHCl_3$ extract of the aqueous filtrate a small amount of purprogenin was obtained.

The aqueous layer was treated by the procedure similar to that for crystal D and the sugar moiety of crystal E was obtained as a colorless syrup.

Rf (BuOH-AcOH-H_2O=4:1:1) :	Sugar of crystal E	0.65
	d-Digitoxose	0.65

The number of digitoxose molecule contained in crystal E molecule was determined by the method as described above.

Sample (γ)		Digitoxose (γ)	Optical density	Content of digitoxose	
				Calcd. for 3 mol.	Found
Crystal E	30.40	16.48	0.433	51.85	54.21

The authors wish to express their appreciations to Dr. K. Takeda, Director of this Laboratory, for his unfailing guidance, and to Prof. M. Tomita and Prof. S. Uyeo of the University of Kyoto for their valuable advises. They would like to thank Mr. T. Wada for the determination of the contents of digitoxose, to Drs. T. Kubota, Y. Matsui and to Messrs. K. Miyahara, T. Ieki, and S. Inaba for microanalyses, ultraviolet and infrared spectra, and optical rotations, to the pharmacological section of this laboratory for the animal tests, and to the members of the Akoh Plant of this firm for the digitalis materials.

Summary

Three new digitanol glycosides (digipronin, purpnin, and purpronin) were isolated from the water-soluble fraction of the leaves of *Digitalis purpurea* L. On hydrolysis, digipronin gave three aglycones (α-, β- and γ-digiprogenin) and one molecule of *d*-digitalose, and purpnin and purpronin gave purpnigenin and purprogenin respectively together with three molecules of *d*-digitoxose. It is likely that these aglycones belong to a C_{21}-steroid of pregnenolone type.

A method for distinguishing the diginigenin type and the pregnenolone type of digitanol glycosides was also described.

(Received February 6, 1961)