Ethyl 4-Cyanophenylsulfonylacetate (XXXVI)—Two g. of ethyl chloroacetate was added to a solution of 4 g. of sodium 4-cyanophenylsulfinate in 20 cc. of water and the mixture was refluxed for 3 hrs. Recrystallization of the separated crystals from EtOH yielded colorless needles of m.p. 70°. Yield, 4 g. *Anal.* Calcd. for $C_{11}H_{11}O_4NS$: N, 5.53. Found: N, 5.39.

4-Formylphenylsulfonylacetic Acid (XXXIV)—To a solution of 5 g. of 4-methylphenylsulfonylacetic acid in a mixture of 20 cc. of Ac_2O and 5 cc. of conc. H_2SO_4, 4 g. of CrO_3 dissolved in 8 cc. of Ac_2O was dropped in under stirring at 10~15°. After the reaction ceased the mixture was poured into ice water, the separated oily substance was heated with 20 cc. of 10% HCl for a while, and crystals that separated after cooling were recrystallized from EtOH to colorless plates, m.p. 150~152°. Yield, 3.5 g. *Anal.* Calcd. for $C_9H_8O_5S$: C, 47.38; H, 3.53. Found: C, 47.24; H, 3.83.

4-Hydroxyiminomethylphenylsulfonylacetic Acid (XXXV)—Prepared from (XXXIV), $NH_2OH \cdot HCl$, and NaOH. Recrystallization from water yielded colorless needles of m.p. 205°(decomp.). *Anal.* Calcd. for $C_9H_9O_5NS$: N, 5.76. Found: N, 5.85.

4-Diacetylaminophenylsulfonylmethane (VII)—4-Acetamidophenylsulfonylmethane (VI) of m.p. 190° was converted to diacetyl derivative by refluxing with Ac_2O for several hrs. After distillation of Ac_2O in vacuum, MeOH and water were added and separated crystals were recrystallized from dil. MeOH to colorless needles of m.p. 152~153°. *Anal.* Calcd. for $C_{11}H_{13}O_4NS$: C, 51.76; H, 5.13. Found: C, 51.87; H, 5.26.

When dil. NaOH was added to a solution of (VII) in EtOH and kept at room temperature, (VI) separated as crystals of m.p. 190°.

4-Diacetylaminomethylphenylsulfonylmethane (XIII)—Prepared from (XII) similarly as above. Recrystallization from dil. MeOH gave colorless needles of m.p. 140°. *Anal.* Calcd. for $C_{12}H_{15}O_4NS$: N, 5.49. Found: N, 500.

Summary

Infrared spectra of 50 phenylsulfonyl derivatives were measured and substituent effect on the sulfonyl group was discussed. Inductive and mesomeric effects of substituents attached to the phenyl ring or directly to the sulfonyl group somewhat affected the frequencies of asymmetric and symmetric stretching vibrations of SO_2. The frequency shifts had some correlations with Hammett's σ-value of the chemical shifts of benzene derivatives. Synthesis of some phenylsulfonyl derivatives is also described.

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UDC 547·918 : 582.572.7

81. Masaharu Yamagishi and Isao Nakamura: Studies on Determination of Sapogenins in Plants belonging to Dioscorea growing in Japan. II.[1] Determination of Diosgenin, Tokorogenin, and a New Genin contained in the Roots.

(*Research Laboratories, Takeda Pharmaceutical Industries, Ltd.**)

In Part I of this series,[1] the authors announced that a new color reaction of diosgenin and tokorogenin satisfies Beer's law and is available for colorimetric determination of these genins. In the present work a method for determining the sapogenins present in roots of *Dioscorea Tokoro* MAKINO and *D. nipponica* M. was established utilizing the above color reaction. As a new genin was found in the sapogenin extract of *D. Tokoro* M. (X-substance**), the present method was studied centering on the separatory determination of diosgenin, tokorogenin, and the X-substance. The main procedures of this method are as follows:

1) Part I: Yakugaku Zasshi, **78**(1958) in press.

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** Morita in this Laboratories found that this substance is identical with yonogenin, m.p. 238~240°, $[\alpha]_D$ −63° (cf. K. Takeda, *et al.*: Yakugaku Zasshi, **77**, 822(1957)).

Powdered sample → extraction of saponins → hydrolysis → extraction of sapogenins → separatory adsorption → colorimetry.

Theoretically, 10 mg. of a dried sample containing about 1% (maximum content is several %) of the genins is enough judging from the sensibility of the color reaction, but the present method was devised to use 100 mg. of a sample for convenience of treatment of the sample and for other reasons.

As no method of this kind has been established, the present method was first applied to the determination of diosgenin in *D. Tokoro* M. growing in Sado Island and the result was compared with the known value obtained by a gravimetric method. Both values were in close agreement.

Mothod

1) Reagent—i) Reagent A : A solution of 26 g. of $SbCl_3$(b.p. 225°), purified by distillation, dissolved in 5 cc. of nitrobenzene was stored, protected from moisture, and diluted with 1/10 its volume of 50% MeOH immediately before use.

ii) Reagent B : $SbCl_3$(b.p. 225°) purified by distillation was mixed with melted phenol in a ratio of 4:5 by weight.

iii) Ethanolic HCl : 2.5*N* HCl was mixed with EtOH in a ratio of 4:5 by volume.

iv) $CHCl_3$(for extraction) : A commercial product containing 0.5-1% of MeOH.

v) $CHCl_3$(for elution) : 1% of EtOH was added to $CHCl_3$ purified by distillation.

vi) Methylene chloride : A commercial preparation was washed with K_2CO_3 solution and water, and a fraction distilling at 40~41° was collected.

vii) Benzene : A commercial product of high quality.

viii) Florisil : 60/100 mesh, produced by Floridin Co.

2) Apparatus—i) Spectrophotometer : Beckman B type.

ii) Adsorption column : A portion of 1 g. of Florisil was poured with benzene into the tubular part of a glass apparatus shown in Fig. 1.

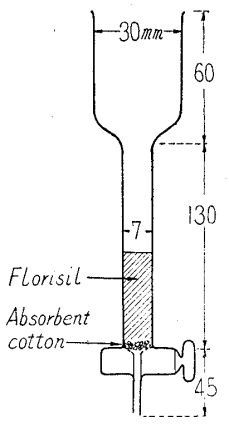


Fig. 1.
Adsorption Apparatus

3) Operation—i) Extraction of Crude Sapogenins : Dried roots were powdered finer than 50 mesh. A mixture of 0.1 g. of the powder in 20 cc. of MeOH was heated in a 50-cc. flask under reflux for 1 hr. on a water bath. After cool, the mixture was filtered through a filter paper and the precipitate on the filter was washed with MeOH. The filtrate was combined with the washing and MeOH was distilled off on a water bath. The residue was hydrolyzed by boiling under reflux with 15 cc. of ethanolic HCl for 3~4 hrs. and the hydrolyzate solution, after being neutralized, was concentrated to remove EtOH. The residue was shaken 3 times with 5-cc. portions of $CHCl_3$ in a separatory funnel to extract the crude genins and the residue obtained by distilling off $CHCl_3$ from the extract almost completely was dissolved in benzene to make up a solution of 10 cc.

ii) Separation of Sapogenins : A 1-cc. portion of the above benzene solution (corresponding to 1/10 of the sample) was poured into the adsorption column to be adsorbed on Florisil. The Florisil was washed with 10 cc. of benzene to remove impurities and eluted first with a mixture of $CHCl_3$ and benzene (8:2 by volume) containing 1% of EtOH to collect 15 cc. of the eluate (eluate A — diosgenin), then with methylene chloride containing 1.5% of EtOH to collect 20 cc. of the eluate (eluate B — X-substance), and finally with methylene chloride containing 7% of EtOH to collect 15 cc. of the eluate (eluate C — tokorogenin).

iii) Colorimetric Determination of Sapogenins: The eluates A, B, and C were evaporated to remove the solvent, and 2 cc. of the reagent A was added to the residue of diosgenin and 2 cc. of the reagent B to each of the residues of X-substance and tokorogenin. These were each heated for a time (diosgenin, at 60° for 20 mins.; X-substance and tokorogenin, at 100° for 60 mins.). After cooling the colored solutions with water, their optical densities (E) at their absorption maxima (diosgenin, 500 mμ; X-substance, 500 or 560 mμ; tokorogenin, 560 mμ) were measured. The quantity of the genin in each eluate was calculated by the equation shown below from the optical density (E_0) obtained by treating in the same way 1 cc. of a benzene solution (100 γ/cc.) of the pure crystals of each genin*** and from the blind test value (B).

$$\text{Quantity of genin} = \frac{100(E-B)}{E_0-B}\gamma$$

Hence, the quantity of genin in 0.1 g. of the material is

$$\frac{100(E-B)}{E_0-B} \times 10 \times \frac{1}{100000} \times 100\%$$

That is,

$$\frac{E-B}{E_0-B}\%$$

4) **Content of the Genins in Dried Root**: 172 samples were determined by the present method and a part of the results is listed in Table I. As seen in the Table, contents of 2% of diosgenin, 1% of X-substance, and 1% of tokorogenin are rather high. In general, a matertial containing diosgenin in a low yield contains X-substance and tokorogenin in high content, and *vice versa*.

Table I. Genin Content of Sample

Sample	Sex	Diosgenin	X-Substance	Tokorogenin
1	♂	0.03%	0.96%	0.68%
2	♂	1.92	0.20	0.33
3	♂	1.34	0.22	0.34
4	♂	2.07	0.20	0.33
5	♂	0.02	0.67	0.79
6	♂	0.14	0.67	0.66
7	♂	0.19	0.79	0.71
8	♂	0.22	0.72	0.84
9	♂	0.15	0.49	0.74
10	♀	0.07	0.76	1.00
11	♀	0.01	1.18	1.07
12	♀	0.13	0.98	0.92
13	♀	0.02	1.12	1.03
14	♀	0.12	0.66	1.03
15	♀	1.26	0.20	0.27
16	♀	0.09	0.45	0.82
17	♀	0.01	0.81	1.06
18	♀	0.09	0.49	0.92
19	♀	0.17	0.67	0.66
20	♀	1.27	0.22	0.33
21	♀	0.11	0.52	0.76
22	♀	0.09	0.93	0.68
23	♀	0.15	0.69	0.84
24	♀	0.11	0.81	0.84
25	♀	0.15	0.74	0.76
26	♀	0.13	0.67	0.83
27	♀	0.16	0.64	0.62
28	♀	0.10	0.62	0.63
29	♀	0.12	0.76	0.60
30	♀	0.02	0.84	0.92

Discussion

1) Hydrolysis—Following the method of Marker[2] the saponins were first extracted and then hydrolyzed by ethanolic HCl. In this case, if the hydrolysis is conducted without EtOH, formation of the sapogenins was incomplete, probably because the sapogenins formed covered the sapogenins. The optimal concentration of HCl and the optimal heating time in the hydrolysis of diosgenin with the same material were as shown in Figs. 2 and 3.

The optimal conditions in the hydrolysis of X-substance and tokorogenin are now

*** m.p.(uncorr.): diosgenin, 205~207°; X-substance, 234~237°; tokorogenin, 266~268°.

2) K.E. Marker: J. Am. Chem. Soc., **69**, 2167, 2230(1948).

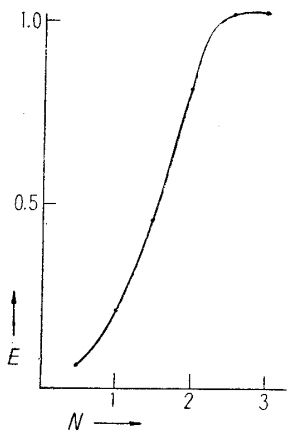


Fig. 2. Effect of HCl-concentration used in HCl-EtOH Mixture

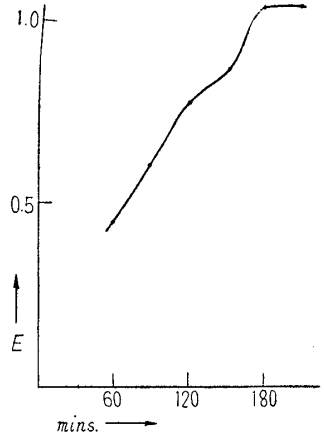
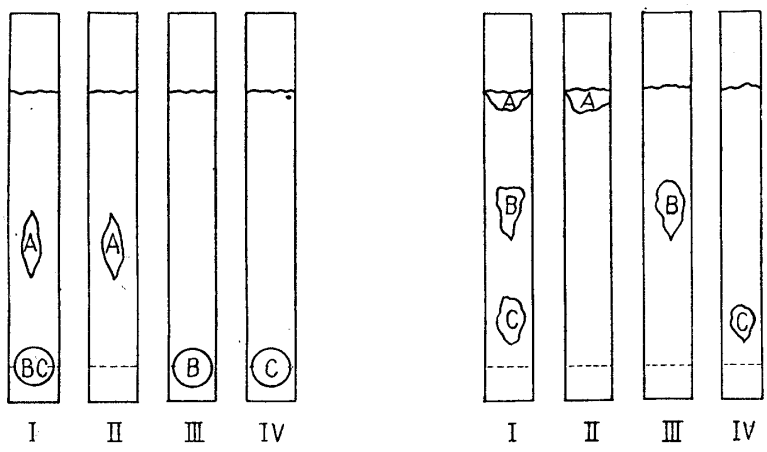


Fig. 3. Effect of Heating Time

under study, but in the present method the hydrolysis was effected by heating in a boiling water bath for 3 hrs.

2) Treatment of Florisil—The sapogenin solutions obtained by eluting the Florisil were investigated by paper partition chromatography. As a result, it was found that each eluate contained a single sapogenin as shown in Fig. 4, indicating that the separation was complete.



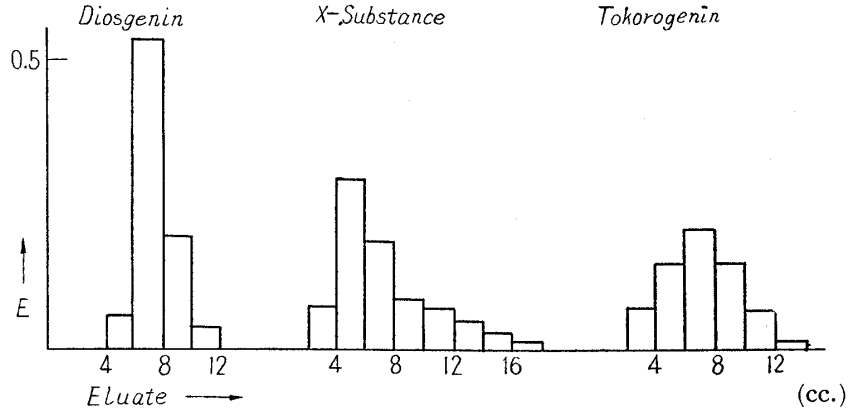
Ligroine-ethanol-water (200:5:45) Ligroine-butanol-ethanol-water (20:1:5:5)

Fig. 4. Chromatogram of Genin Solution Before and After Purification
I. Before purification by Florisil
II. Eluate by $CHCl_3$ (1% EtOH)-benzene (8:2 vol)
III. Eluate by CH_2Cl_2 (1.5% EtOH)
IV. Eluate by CH_2Cl_2 (7% EtOH)
A···diosgenin B···X-substance C···tokorogenin

On the other hand, a benzene solution containing ca. 100 γ of diosgenin and ca. 200 γ each of X-substance and tokorogenin was adsorbed on Florisil, and each of the genins was eluted with a proper solvent. The state of elution of each genin was investigated by coloration of the eluate to give a histogram shown in Fig. 5.

3) Result—There is no means to give a direct evidence that the value obtained by the present method is correct. However, the value of diosgenin obtained by this method from *Dioscorea Tokoro* MAKINO growing in Sado Island was in close agreement with the value obtained by a gravimetric determination by Inoue[3] of the same sample, as shown in Table II.

3) M. Inoue : Japan. Pat. 207776(1954).



$CHCl_3$ (1% EtOH)-benzene (8:2) CH_2Cl_2 (1.5% EtOH) CH_2Cl_2 (7% EtOH)

Fig. 5. Histogram of Elution

Table II. Comparison of the Values

Sample	By Inoue's method (%)	By this method (%)
1	2.00	2.04
2	1.13	1.21
3	0.34	0.36
4	2.22	2.43

From the result in Table II and from those mentioned in 1) and 2), the present method for determing diosgenin, X-substance, and tokorogenin in the sample seems to be fully reliable.

The authors wish to express their grateful thanks to Dr. Satoru Kuwada and Dr. Atsushi Watanabe for their encouragement throughout the present work and to Dr. Masamoto Nishikawa for his kindness in supplying the sapogenin crystals.

Summary

The alcoholic extract of roots of Dioscorea growing in Japan was hydrolyzed with hydrochloric acid and the resulting mixture of sapogenins was separated into pure diosgenin, tokorogenin, and yonogenin by adsorption chromatography on Florisil. Each of the products was determined colorimetrically with reagents containing antimony trichloride as the chief component.

(Received March 28, 1958)

UDC 545.33 : 547.655.6

82. Morizo Ishidate, (the Late) Takashi Isshiki, and Keizo Tada : Nonaqueous Polarography of Quinones. V.* Half-wave Potentials of *o*-Quinones in Relation to Carcinogenesis of Polyaromatic Hydrocarbons.

(*Faculty of Pharmaceutical Sciences, University of Tokyo***)

Various hypotheses or theoretical treatment have recently been developed for evaluation of the mechanism of carcinogenesis of polyaromatic hydrocarbons, but keys to solving this troublesome problem have not yet been provided by any of these speculations. It seems rather strange that none of them have referred to the probable rôle of quinones, some of which have recently been proved to result from hydrocarbons in a living body, in spite of the fact that *p*-benzoquinone and 1,4-naphthoquinone have some

* Part IV : This Bulletin, **3**, 312(1955).

** Hongo, Tokyo (石館守三, (故)一色 孝, 多田敬三).