washed with 5 cc. of glacial AcOH. To the combined filtrate and washing, 30 cc. of water was added and the separated substance was recrystallized from glacial AcOH to light yellow needles, m.p. 207° (decomp.). Yield, 40 mg. *Anal.* Calcd. for $C_{53}H_{42}O_6$: C, 82.15; H, 5.46; CH_3CO, 16.65. Found : C, 82.67; H, 5.34; CH_3CO, 16.5.

Absorption spectra—The ultraviolet spectra were measured by a Shimadzu Spectrophotometer type QB-50 in a cell of 10 mm. optical depth with the reagent blank. The infrared spectra were measured by a Perkin-Elmer Infrared Spectrophotometer in Nujol mull with about 0.01 mm. thickness.

Summary

The anthrone color of glucose was shown to have the maximum intensity when the reaction was carried out for 20 minutes at 85~90° in 70%(v/v) of sulfuric acid. The absorption maximum of the blue-green color shifted to a longer wave length region with the increasing concentration of the acid. It seemed to require three moles of anthrone to one mole of glucose in the reaction.

The soluble part of coloring matter of the reaction in benzene was separated on alumina and was shown to consist of several dyes which gave different coloration with sulfuric acid. One of the main dyes was isolated in crystalline form, and its probable structure might be 1,2,5- or 1,3,5-trianthronylidenepentane from its analytical data and infrared spectrum, and its reaction mechanism was considered. The same dye was also isolated from anthronated xylose and the reaction mechanism with the pentose was discussed.

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9. **Masao Shimizu, Genkichi Ohta, Shin-ichi Kitahara,* Genzo Tsunoo, and Shin-ichiro Sasahara** : Studies on the Constituents of Rice Bran Oil. I. Isolation of Phenolic Substances.

(*Yanagishima Research Laboratory,* and Hirai Factory,** Daiichi Seiyaku Co., Ltd.*)

During their studies on vegetable oils, Tsuchiya and Kaneko[1] found that rice bran oil exhibited a characteristic absorption in the ultraviolet region and searching for its source, isolated a crystalline substance, which they named oryzanol. Accoring to their report, oryzanol melts at 137.5~138.5°, shows absorption maxima (in heptane) at 231, 290 and 315 mμ; colors yellow with alkali hydroxides, is negative to sterol color reactions, and presence of a hydroxyl and carbonyl groups is suggested from its infrared absorption spectrum.

The present writers attempted the extraction of this substance from rice bran oil, through the kind permission of the foregoing workers, and a few observations were gained, which are reported in the present paper.

Tsuchiya and Kaneko removed free fatty acids from rice bran oil with sodium carbonate, esterified the fats, and fatty acid ester were removed by low-pressure distillation. Repeated chromatography of the residue and recrystallization afforded oryzanol.

Under the detailed instructions of the foregoing workers, oryzanol was successfully

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* Hirakawabashi, Sumida-ku, Tokyo (清水正夫，太田元吉，北原琛一).

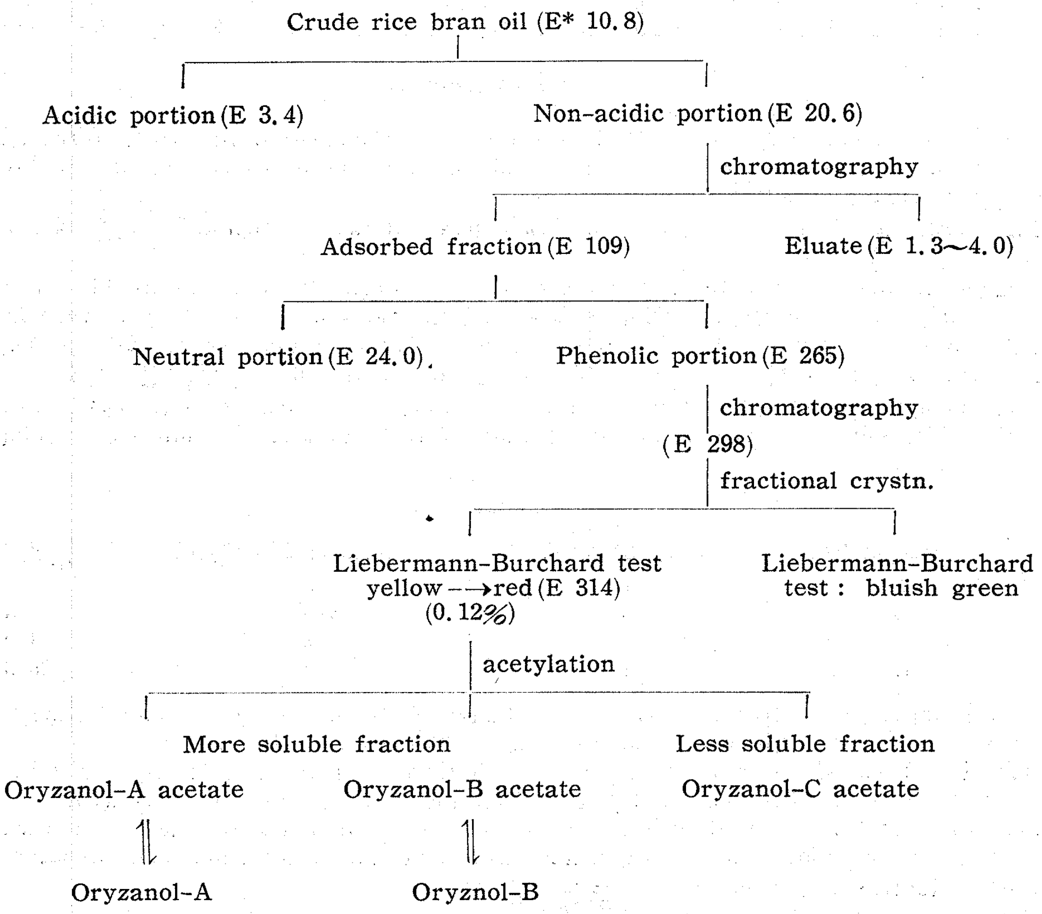
** Hirai-cho, Edogawa-ku, Tokyo (角尾元三，笹原慎一郎).

[1] R. Kaneko, T. Tsuchiya : J. Soc. Chem. Ind. Japan, **57**, 526(1954).

isolated by the same method and, as will be described in the following paper,[2] the hydroxyl in this substance was found to be phenolic. Therefore, using this nature, a different method of extraction was attempted.

Removal of free fatty acids from crude rice bran oil can best be effected with 40% sodium carbonate (supersaturated) in dichloroethane solution and the procedure is the easiest. Next, deacidified oil is dissolved in petroleum ether and this solution is passed through an alumina column to allow adsorption of phenolic substances. The column is eluted with a mixture of glacial acetic acid and benzene, the eluted phenolic substance as an ether solution is transited to 2% potassium hydroxide solution, to remove non-phenolic substances, and the benzene solution is again passed through an alumina column. On elution of this column with a mixture of ether and methanol, an ointment-like substance of $\lambda^{\mathrm{EtOH}}_{322\,\mathrm{m}\mu}$ $E^{1\%}_{1\mathrm{cm}}$ 298 is obtained. Fractional recrystallization of this substance from ether and ethanol separates it into sparingly soluble substance of m.p. 125~143°, $\lambda^{\mathrm{EtOH}}_{322\,\mathrm{m}\mu}$ $E^{1\%}_{1\mathrm{cm}}$ 314, giving yellow to red coloration in Liebermann-Burchard reaction, and an easily soluble substance that gives bluish green coloration in the Liebermann-Burchard reaction.

Acetylation of the sparingly soluble substance with acetic anhydride and pyridine and recrystallization from acetic ester separated it into three substances by difference of solubility. The most easily soluble substance, m.p. 186~187°, $[\alpha]_D$ +39.5°, on deacetylation afforded a substance of m.p. 151°, $[\alpha]_D$ +40°, $\lambda^{\mathrm{EtOH}}_{322\,\mathrm{m}\mu}$ $E^{1\%}_{1\mathrm{cm}}$ 357, which was designated oryzanol-A. Deacetylation of the sparingly soluble substance, m.p. 204~206°,



* $E = \lambda^{\mathrm{EtOH}}_{322\,\mathrm{m}\mu}$ $E^{1\%}_{1\,\mathrm{cm}}$

Chart 1.

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[2] G. Ohta, M. Shimizu : This Bulletin, **5**, 40(1957).

$[\alpha]_D$ +42°, gave a substance of m.p. 150°, $[\alpha]_D$ +45°, $\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm}$ 340, which was designated oryzanol-B. The most sparing in acetic ester, an acetate of m.p. 216~217°, obtained in a minute amount, was designated as oryzanol-C acetate. The yield of the extraction against the crude rice bran oil was 0.12% of a mixture of m.p. 125~143°, or after acetylation, fractional recrystallization, and deacetylation, 0.04% of oryzanol-A and 0.035% of oryzanol-B (cf. Chart 1).

The so-separated oryzanol-A and -B possess extremely similar properties but show marked depression of the melting point on admixture. Their acetates have different melting points and their admixture gives intermediate value. Since the acetylation of oryzanol-A gives only the oryzanol-A acetate and same is true of oryzanol-B, it cannot be assumed that they were formed by isomerization during isolation from their mixture through the acetates. As will be described in more detail in the following paper,[2] oryzanol-A is clearly different from -B and both are thought to be present in natural state.

As shown above, the substance present in rice bran oil responsible for the characteristic absorption in the ultraviolet range and detected by Tsuchiya and Kaneko is not a single unity but was separated into three substances, oryzanol-A, -B, and -C. The earlier workers also seemed to have entertained the idea that their oryzanol was a mixture of substances, according to private communication.

Deep gratitude is tendered to Dr. Tomotaro Tsuchiya of the Tokyo Technological Laboratory for offering unpublished data and giving many kind and valuable advices. The writers are grateful to Dr. Junzo Shinoda, President of this Company, for kind guidances during the course of this work, to Mr. Nakano, Director of the Yanagishima Factory, and to Mr. Obika, Director of the Hirai Factory, for giving them facilities, and to Messrs. M. Negishi, K. Abe, and S. Kawagoe for elemental analyses.

Experimental

All m.p.s are not corrected. Optical rotation was measured in $CHCl_3$, ultraviolet absorption was measured in the range above 220 mμ, and the absorption maximum of rice bran oil shifted from $\lambda^{heptane}_{max}$ 315 mμ to λ^{EtOH}_{max} 322 mμ.

Raw Material—Crude rice bran oil used had following constants: λ^{EtOH}_{max} 322 mμ ($E^{1\%}_{1cm.}$ 10.8); $\lambda^{heptane}_{max}$ 315 mμ ($E^{1\%}_{1cm.}$ 10.5).

Deacidification—To a solution of 2 kg. of the rice bran oil dissolved in 4 L. of dichloroethane, 2.5 L. of 40% Na_2CO_3(supersaturation) was added, the fatty acid salts formed were filtered off with pressure, and washed with 2 L. of dichloroethane. The combined dichloroethane solution afforded 730 g. of greenish oil, $\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm.}$ 20.6. The fatty acid liberated: $\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm.}$ 3.4.

Chromatography—A solution of the foregoing 730 g. of a substance dissolved in 1 L. of petr. ether was passed through a column containing 1.8 kg. of Al_2O_3 and the column was developed with petr. ether (cf. Table I).

Table I.

Fraction No.	Petr. ether (L.)	Eluate residue (g.)	$\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm}$
I	1	0	
II	2	510	1.3
III	1	78	4.0
IV	1	8	28.0

The alumina was extracted with 4 L. of glacial AcOH-benzene (1 : 3) and afforded 103 g. of a substance, $\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm.}$ 109, which colored green with $FeCl_3$ solution in EtOH.

Alkali Extraction—A solution of the foregoing 103 g. of extract dissolved in 2.5 L. of ether was shaken with a total of 400 cc. of 2% KOH, the aqueous layer was washed with 1 L. of ether, and acidified with AcOH. The oil that separated out was extracted with ether, which was washed with 30% K_2CO_3 solution, and the ether evaporated. Yield of the residual phenolic substance, 28 g., $\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm.}$ 265. Neutral substance, 63 g., $\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm.}$ 24.

Chromatography—A solution of 28 g. of the foregoing phenolic substances dissolved in 600 cc. of benzene was passed through a column (5.4×37 cm.) of 800 g. of Al_2O_3 and the column was developed as shown in Table II.

The alumina, excluding the top 5 cm., was extracted with 4 L. of a mixture of benzene-glacial AcOH(10 : 1) and 2.9 g. of a substance, $\lambda^{EtOH}_{322\,m\mu}$ $E^{1\%}_{1cm.}$ 196, was obtained.

TABLE II.

Fraction No.	Eluant (L.)	Eluate residue (g.)	$\lambda_{322\,\mathrm{m}\mu}^{\mathrm{EtOH}}$ $E_{1\mathrm{cm.}}^{1\%}$
I	Benzene-ether (10 : 1) 3 Benzene-ether (1 : 1) 2.5	1.0	3.2
II	Ether 3 Ether-MeOH (10 : 1) 1	2.6	3.9
III	Ether-MeOH (10 : 1) 10.5	16.6	298

Fractional Recrystallization—The foregoing 16 g. of Fraction III was fractionally recrystallized from ether-EtOH. The sparingly soluble portion : 2.42 g. of m.p. 125~143°; $\lambda_{322\,\mathrm{m}\mu}^{\mathrm{EtOH}}$ $E_{1\mathrm{cm.}}^{1\%}$ 314, giving yellow to red coloration in the Liebermann-Burchard reaction. The easily soluble portion : 11.3 g. of a substance giving bluish green coloration in the Liebermann-Burchard reaction.

Acetylation—The foregoing sparingly soluble portion (2.42 g.) was acetylated by allowing to stand with Ac_2O and pyridine at room temperature over night and 2.5 g. of a substance melting at 190~197° was obtained. Repeated fractional recrystallization from AcOEt afforded 3 kinds of crystals. The easily soluble portion crystallized from AcOEt-EtOH to prisms or needles, m.p. 186~187°, $[\alpha]_D$ +39.5°(c=1.04). Yield, 950 mg. *Anal.* Calcd. for $C_{42}H_{60}O_5$(Oryzanol-A acetate) : C, 78.22; H, 9.38; CH_3CO, 6.68. Found : C, 78.30; H, 9.44; CH_3CO, 6.74.

The sparingly soluble portion crystallized from benzene-AcOEt to needles, m.p. 204~206°, $[\alpha]_D$ +42°(c=1.12). Yield, 820 mg. *Anal.* Calcd. for $C_{43}H_{62}O_5$(Oryzanol-B acetate) : C, 78.38; H, 9.48. Found : C, 78.11, 78.13; H, 9.11, 9.48.

Mixed fusion of the acetates of oryzanol-A and -B, m.p. 195~199°, not showing clear depression of m.p.

The most sparingly soluble portion crystallized from benzene-AcOEt to needles, m.p. 216~217°, designated as oryzanol-C acetate.

Oryzanol-A—A mixture of 900 mg. of oryzanol-A acetate, 10 cc. of benzene, and 90 cc. of 0.1*N* KOH-MeOH was warmed at 50° for 1 hr. and neutralized with glacial AcOH. The product was recrystallized from benzene-EtOH and air-dried to m.p. 150°(efferv. at 120~125°). *Anal.* Calcd. for $C_{40}H_{58}O_4 \cdot 2H_2O$: $2H_2O$, 5.64; $1H_2O$, 2.82. Found : $2H_2O$(Karl-Fischer), 5.9; $1H_2O$(dried), 3.04.

This was dried at 60° for 4 hrs. *in vacuo* : m.p. 150.5~151.5°, $[\alpha]_D$ +40°(c=0.68). U.V. $\lambda_{\mathrm{max}}^{\mathrm{EtOH}}$: 322 mμ(log ε 4.34); $\lambda_{\mathrm{max}}^{\mathrm{heptane}}$ mμ(log ε) : 231 (4.15), 290 (4.24), 315 (4.34). *Anal.* Calcd. for $C_{40}H_{58}O_4 \cdot H_2O$: C, 77.37; H, 9.74; CH_3O, 5.00; H_2O, 2.90. Found : C, 77.10, 76.97; H, 9.88, 9.69; CH_3O, 5.29; H_2O (Karl-Fischer), 3.06. The one mole of water could not be removed even on drying at 100° for 24 hrs. in high vacuum.

Oryzanol-A is recovered on warming with 0.1*N* KOH-EtOH for 30 mins. It colors green with $FeCl_3$ in EtOH, reddish violet with diazobenzenesulfonic acid, and yellow to red in the Liebermann-Burchard reaction, with green fluorescence. Acetylation of this substance gave an acetate of m.p. 185.5~187°, undepressed on admixture with the earlier acetate.

Oryzanol-B—A mixture of 810 mg. of oryzanol-B acetate, 10 cc. of benzene, and 80 cc. of 0.1*N* KOH-MeOH was warmed at 40° for 1 hr. and treated as in the case of oryzanol-A. Recrystallization from $CHCl_3$-MeOH yielded 740 mg. of plates, m.p. 150°, $[\alpha]_D$ +45°(c=1.19); U.V. $\lambda_{\mathrm{max}}^{\mathrm{EtOH}}$: 322 mμ (log ε 4.32); $\lambda_{\mathrm{max}}^{\mathrm{heptane}}$ mμ(log ε) : 232 (4.17), 290 (4.28), 315 (4.36). *Anal.* Calcd. for $C_{41}H_{60}O_4$: C, 79.82; H, 9.80; CH_3O, 5.04. Found : C, 79.50; H, 9.41; CH_3O, 4.60.

Oryzanol-B colors green with $FeCl_3$ in EtOH, reddish violet with diazobenzenesulfonic acid, and yellow to red coloration with green fluorescence in the Liebermann-Burchard reaction. Mixed fusion with oryzanol-A gave m.p. 137~143°. Acetylation of this substance gave the acetate of m.p. 204~206°, undepressed on admixture with the original acetate.

Summary

Tsuchiya and Kaneko[1] had isolated a substance exhibiting ultraviolet absorption maxima (in heptane) at 231, 290, and 315 mμ from rice bran oil and named it oryzanol. Utilizing the fact that oryzanol is a phenolic substance, the rice bran oil removed of free fatty acids by sodium carbonate in dichloroethane solution was passed through an alumina column and the adsorbed phenolic substance was eluted. This phenolic substance, as an ether solution, was purified by transiting to potassium hydroxide solution and further recrystallized to crystalline oryzanol. This was acetylated and fractionally recrystallized from ethyl acetate, separating into three kinds of substances. The most sparingly soluble portion was in a minute amount but the other two liberated phenolic substances on deacetylation. These were designated as oryzanol-A and -B.

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