U.D.C. 547.915 : 665.323

10. **Genkichi Ohta and Masao Shimizu**: Studies on the Constituents of Rice Bran Oil. II.[1] Structure of Oryzanol-A.

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

It was shown in the preceding paper[1] that oryzanol-A, -B, and -C were isolated as the constituents responsible for characteristic absorption maxima in the ultraviolet range in rice bran oil. The structure of oryzanol-A was later established and few observations were gained of oryzanol-B, which are described herein.

Oryzanol-A comes as crystals which effervesce at 120~125° and melt at 150°, possessing two moles of the water of crystallization as determined by the Karl Fischer method. Drying in vacuum liberates one mole of water to form crystals of m.p. 150.5~151.5°, $[\alpha]_D$ +40°. The Liebermann-Burchard reaction is yellow to red coloration and no precipitate is formed with digitonin. It is sparingly soluble in alkali hydroxides but transits to the alkali hydroxide layer when its ether solution is shaken with alkali solution, which colors yellow and red with diazobenzenesulfonic acid. Its ethanol solution colors green with ferric chloride. It forms an acetate of m.p. 186~187°, $[\alpha]_D$ +39.5°. While it forms impurifiable substance with diazomethane in excess, it is methylated with dimethyl sulfate to give a crystalline methyl ether, m.p. 142~143°, $[\alpha]_D$ +40°. These properties indicate the presence of a phenolic hydroxyl group and the presence of a methoxyl group in oryzanol-A had been indicated by the Zeisel method.

Oryzanol-A acetate is extremely easily deacetylated, even with alumina. The acetate is deacetylated when warmed with 0.1*N* potassium hydroxide-ethanol solution for 30 minutes but oryzanol-A itself remains inert under these conditions and the starting material is recovered. Oryzanol-A methyl ether is saponified when warmed with 0.2*N* potassium hydroxide-ethanol and affords a carboxylic acid and a neutral substance. This carboxylic acid, m.p. 181~182°, was established as 3,4-dimethoxycinnamic acid. The molecular weight of the methyl ether was determined by titration according to the report of Sandquist and Gorton[2] and values obtained were 617.4, 619.3, and 619.7. Considering the elemental analytical values of various derivatives, molecular formula of $C_{41}H_{60}O_4$ (mol. wt., 616.9) was assigned for the methyl ether. Oryzanol-A itself is saponified under a more drastic conditions to form ferulic acid and a neutral substance, and the molecular formula of $C_{40}H_{58}O_4 \cdot H_2O$ seemed appropriate for oryzanol-A.

Oryzanol-B also shows exactly the same properties and the molecular weight of its methyl ether was determined as 633 and 628. Except for the fact that the molecular formula of its methyl ether seems more like $C_{42}H_{62}O_4$(mol. wt., 630.9) rather than $C_{41}H_{60}O_4$ (mol. wt., 616.9), oryzanol-B was also established as the ester of ferulic acid.

Since the foregoing data made it clear that oryzanol-A and -B are the esters of ferulic acid, the ultraviolet absorption spectra of ferulic acid, methyl ferulate, and oryrzanol-A and -B were compared and they all were similar. The neutral substance obtained by saponification does not exhibit any absorption above 220 mμ, so that the characteristic absorption of rice bran oil observed by Tsuchiya and Kaneko[3] is now known to be due to ferulic acid.

The neutral substances obtained by the saponification of oryzanol-A and -B color yellow to red (with green fluorescence) by the Liebermann-Burchard reaction, red to

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* Hirakawabashi, Sumidaku, Tokyo (太田元吉, 清水正夫).

[1] Part I: This Bulletin, **5**, 36(1957).

[2] H. Sandquist, J. Gorton: Ber., **63**, 1935(1930).

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

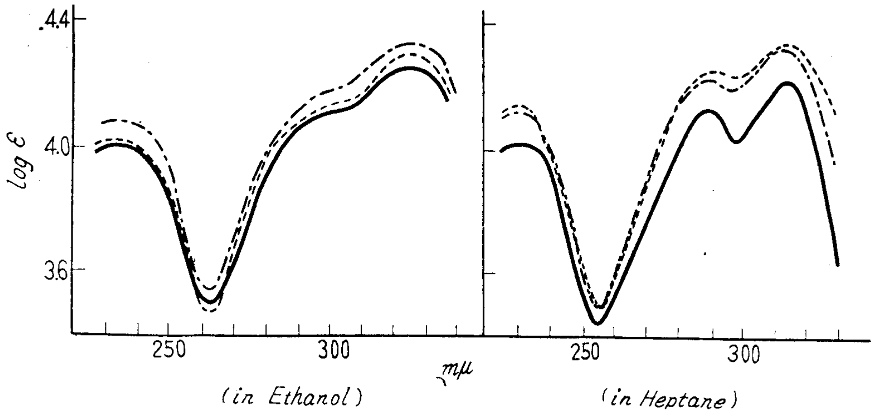

Fig. 1. Ultraviolet Spectra

Tschugaeff reaction, negative to Tortelli-Jaffé reaction, and forms a precipitate with digitonin, but the precipitate is easily soluble in ethanol. The substances form a monoacetate and monobenzoate, and are both triterpene alcohol, which are tentatively designated as alcohol-A and -B. From the molecular formulae of the methyl ethers of oryzanol-A and -B, alcohol-A is assigned the molecular formula of $C_{30}H_{50}O$ and alcohol-B, $C_{30\sim31}H_{50\sim52}O$. Both acetates form a dibromide and the presence of one double bond was indicated by titration with perbenzoic acid. Alcohol-A acetate exhibits absorption in infrared spectrum at 12.1 μ(826 cm^{-1}), suggesting the presence of a trisubstituted double bond ($-CH{=}C<$), and the absorptions at 11.28 μ(887 cm^{-1}) and 6.07 μ(1645 cm^{-1}) in alcohol-B acetate suggest the presence of vinylidene group ($>C{=}CH_2$)(Fig. 2). The acetate dibromide of both A and B still colors pale yellow with tetranitromethane.

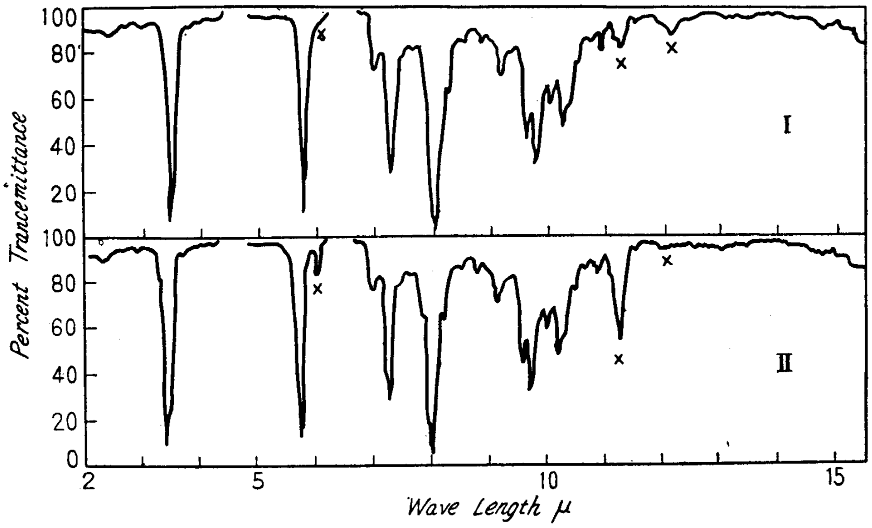

Fig. 2. Infrared Spectra (in CS_2)

I. Cycloartenyl acetate
II. Alcohol-B acetate

Comparison of alcohol-A and its derivatives with the known triterpene alcohols is given in Table I and there is an extreme similarity with cycloartenol. Mixed fusion of the acetate and benzoate of cycloartenol, kindly supplied by Prof. F. S. Spring, with those obtained by the writers established their identity. The cycloartenol ester of ferulic acid was prepared and compared with oryzanol-A and their identity became evident.

The foregoing experiments have shown that oryzanol-A is cycloartenyl ferulate. Suzuki[4] had already reported the presence of ferulic acid in rice bran oil but did not mention that of cycloartenol. The structural determination of cycloartenol was recently made by Barton,[5] Spring,[6] and Cole,[7] as an example of triterpene alcohols possessing

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4) B. Suzuki : J. Chem. Soc. Japan, **45**, 302(1914).
5) D. H. R. Barton : J. Chem. Soc., **1951**, 1444.
6) H. R. Bentley, *et al.* : *Ibid.*, **1953**, 3673; D. S. Irvine, *et al.* : *Ibid.*, **1955**, 1317.
7) A. R. H. Cole : *Ibid.*, **1954**, 3810.

a cyclopropane ring. It has been isolated in natural sources from *Artocarpus integrifolia*,[5,8] *Strychnos Nux vomica*,[6] *Euphorbia balsamifera*,[9] and *Euphorbia handiensis*,[10] but this is the first time that this alcohol had been isolated as an ester.

TABLE I.

	Authors'		Nath[8]		Barton[5]		Chapon[9]		Spring[6]	
	m.p.(°C)	$[\alpha]_D$	m.p.(°C)	$[\alpha]_D$	m.p.(°C)	$[\alpha]_D$	m.p.(°C)	$[\alpha]_J$	m.p.(°C)	$[\alpha]_D$
Enol	114\~115	+52°	106\~107		85\~92	+48°	85\~105	+48°	115	+54°
Enyl acetate	122\~124	+59°	119\~120		122.5\~123.5	+58°	119\~120	+58°	122\~124	+59.5°
Enyl benzoate	129\~130	+75°			129\~130	+65°	130	+67°	130	+76°
Enyl benzoate dibromide	182\~183 (decomp.)		182\~183 (decomp.)							
Enone	108\~109	+24°	109	+23°	109	+24°			105\~106	+22°
Enone semicarbazone	203\~204 (decomp.)		203\~204 (decomp.)							
Enone oxime	175.5\~176.5		175							

TABLE II.

	Enol		Enyl acetate		Enyl benzoate		Enyl *p*-nitrobenzoate	
	m.p.(°C)	$[\alpha]_D$	m.p.(°C)	$[\alpha]_D$	m.p.(°C)	$[\alpha]_D$	m.p.(°C)	$[\alpha]_D$
Alcohol-B	114\~115	+55°	109\~110	+68°	117.5\~118.5	+67°	227\~228	
α-Tritisterol[a]	114\~115	+54.3°*	107\~108	+70.4°				
β-Orysterol[b]	113\~114	+51.9°*	104				227\~228	
Resiniferol[c]	118\~120		107\~109	+60°	127\~129			
β-Balalbaresinol[d]	115\~116		107.5		120\~122.5			
Cyclolaudenol	125	+46°	120\~121	+55°	194\~195	+63°		

* Measured in EtOH Solution.

a) P. Karrer, *et al.* : Helv. Chim. Acta, **20**, 424, 1422(1937).

b) A. R. Todd, *et al.* : Biochem. J. (London), **31**, 2247(1937).

c) G. Dupont, *et al.* : Bull. soc. chim. France, **1953**, 852.

d) N. H. Cohen : Arch. Pharm., **246**, 510(1908).

The melting point and optical rotation of alcohol-B and its derivatives are similar to those of α-tritisterol, among the known triterpene alcohols, as listed in Table II. Since its acetate dibromide colors with tetranitromethane, as with cycloartenol, the presence of a cyclopropane ring could be assumed but the presence of a vinylidene group suggested by the infrared spectrum differs from cycloartenol. Various constants differ from those of cyclolaudenol[11] possessing a vinylidene group, that it is assumed that alcohol-B is a new cyclopropane-triterpene alcohol with a different side chain. The tentative name is retained pending further investigation.

The writers are indebted Prof. F. S. Spring for his kindness in providing them with the sample of cycloartenol, to Mr. Seishi Tanaka, Institute of Technological Research, University of Tokyo, for infrared spectral measurements, to Dr. Junzo Shinoda, President of this Company, for kind guidance, to Mr. Isamu Nakano, Director of the Yanagishima Factory, for giving them facilities, and to Messrs. Minoru Negishi and Katsuji Abe for elemental analyses.

Experimental

Oryzanol-A Methyl Ether—A mixture of 0.77 g. of oryzanol-A, 2.26 g. of K_2CO_3, 1.27 cc. of Me_2SO_4, and 40 cc. of acetone was refluxed for 2 hrs. and the product was recrystallized from a mixture of AcOEt-EtOH to 0.61 g. of needles, m.p. 141\~143°, $[\alpha]_D$ +40° (c=1.24). *Anal.* Calcd. for

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[8] M. C. Nath : Z. physiol. Chem., **247**, 9, 71(1937).

[9] S. Chapon, S. David : Bull. soc. chim. France, **1952**, 456.

[10] A. G. Gonzalez, J. G. Breton : Annea real soc. espan. fis. y quim. (Madrid), **49B**, 223(1953) (C.A., **48**, 7040(1954)).

[11] H. R. Bentley, *et al.* : J. Chem. Soc., **1955**, 596; J. A. Henry, *et al.* : *Ibid.*, **1955**, 1607.

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$C_{41}H_{60}O_4$: C, 79.82; H, 9.80; CH_3O, 10.6; mol. wt., 616.9. Found: C, 79.91; 79.82; H, 9.67, 9.74; CH_3O, 10.20; mol. wt. (titration), 619.3, 617.4, 619.7.

Saponification of Oryzanol-A Methyl Ether—A mixture of 0.726 g. of the methyl ether and 10 cc. of 0.2*N* KOH-EtOH was refluxed for 4 hrs. and titrated with 0.05*N* HCl (indicator: phenolphthalein). Consumption of the acid: 16.24 cc. (control: 39.69 cc.). Mol. wt. Found: 619.3. The titrated solution was acidified with HCl and extracted with ether to be separated into acidic and neutral portions.

The acid portion was recrystallized from benzene to crystals of m.p. 181~182°. *Anal.* Calcd. for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; CH_3O, 29.81. Found: C, 63.15; H, 5.89; CH_3O, 30.09. No depression of the melting point occurred on admixture with 3,4-dimethoxycinnamic acid.

The neutral portion crystallized from EtOH-MeOH to needles, m.p. 102° (sint. 85~95°), which, after drying at 60° for 48 hrs. in high vacuum, melted at 114~115°, $[\alpha]_D$ +49°, +52° (c=1.38, 1.99). This is designated as alcohol-A (cycloartenol). *Anal.* Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.03; H, 11.95.

Saponification of Oryzanol-A—A mixture of 1.2 g. of oryzanol-A and 60 cc. of 10% KOH-EtOH was refluxed for 6 hrs., and the product was separated into neutral and phenolic portions.

The neutral portion yielded 0.7 g. of the same substance as above, m.p. 102°.

The acid portion was recrystallized from hydrous EtOH to 0.4 g. of ferulic acid, m.p. and mixed m.p. 171°; λ_{max}^{EtOH} 322 mμ (log ε 4.22). *Anal.* Calcd. for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19; CH_3O, 15.94. Found: C, 61.63; H, 5.24; CH_3O, 16.04. Acetate: m.p. 196~197°. *Anal.* Calcd. for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12. Found: C, 61.01; H, 5.31.

Methyl Ferulate—A mixture of ferulic acid, MeOH, and conc. H_2SO_4 was boiled for 4 hrs. b.p$_1$ 163°; λ_{max}^{EtOH} 322 mμ (log ε 4.22); $\lambda_{max}^{heptane}$ mμ (log ε): 231 (4.06), 291 (4.13), 314 (4.21). Acetate: m.p. 124°. *Anal.* Calcd. for $C_{13}H_{14}O_5$: C, 62.39; H, 5.64. Found: C, 62.48; H, 5.97.

Cycloartenyl Acetate—The foregoing substance of m.p. 100~102° was acetylated with Ac_2O and pyridine and recrystallized from acetone to crystals of m.p. 122~124°, undepressed on admixture with the sample provided by Prof. F. S. Spring. $[\alpha]_D$ +59° (c=2.62). I.R. $\nu_{max}^{CS_2}$ 826 cm^{-1} (-CH=C<). *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 82.29; H, 11.06.

Dibromide: CCl_4 solution of Br_2 was added to CCl_4 solution of the foregoing acetate, under ice cooling, until the solution acquired the color of bromine. After evaporation of the solvent under a reduced pressure, the residue was recrystallized from ether-acetone mixture to plates, m.p. 176~177°, $[\alpha]_D$ +16° (c=0.75). *Anal.* Calcd. for $C_{32}H_{52}O_2Br_2$: C, 61.14; H, 8.34. Found: C, 61.43; H, 8.07.

The dibromide colored pale yellow with tetranitromethane and its debromination with Zn-AcOH regenerated the acetate of m.p. 122~124°.

Cycloartenyl Benzoate—Obtained by the application of BzCl to cycloartenol in pyridine and recrystallized from acetone-EtOH to crystals of m.p. 129~130°, undepressed on admixture with the sample provided by Prof. F. S. Spring. $[\alpha]_D$ +75° (c=1.61). *Anal.* Calcd. for $C_{37}H_{54}O_2$: C, 83.72; H, 10.25. Found: C, 83.94; H, 10.01.

A mixture of 0.2360 g. of the benzoate and $CHCl_3$ solution of 0.42 mole of perbenzoic acid was allowed to stand in an ice chamber and consumption of oxygen determined by titration was 1.13 moles after 16 hrs., 1.19 moles after 32 hrs.

Dibromide: The benzoate was brominated in CCl_4 with CCl_4 solution of Br_2 under ice cooling and the product was recrystallized from AcOEt to plates, m.p. 182~183° (decomp.), $[\alpha]_D$ +21° (c=0.52). *Anal.* Calcd. for $C_{37}H_{54}O_2Br_2$: C, 64.35; H, 9.28. Found: C, 64.27; H, 8.32.

Cycloartenyl *p*-Nitrobenzoate—A mixture of cycloartenol and *p*-nitrobenzoyl chloride in pyridine was heated at 100° for 1 hr. and recrystallized from AcOEt-benzene to needles, m.p. 216~217°, $[\alpha]_D$ +65.3° (c=1.08). *Anal.* Calcd. for $C_{37}H_{53}O_4N$: C, 77.17; H, 9.28. Found: C, 77.18; H, 9.02.

Cycloartenone—A mixture of 0.42 g. of cycloartenol, 1.5 cc. of cyclohexanone, and 20 cc. of toluene was warmed, 10 cc. of the solvent was distilled off, 0.6 g. of Al *tert*-butoxide added, and the mixture was refluxed for 6 hrs. To this was added 2% NaOH solution and extracted with benzene. The benzene residue was dissolved in petr. ether, passed through a column of 16 g. of alumina, and the column eluted with petr. ether-benzene (9 : 1). The residue from this eluate (0.32 g.) was recrystallized from $CHCl_3$-MeOH to crystals of m.p. 108~109°, $[\alpha]_D$ +24° (c=0.91). *Anal.* Calcd. for $C_{30}H_{48}O$: C, 84.81; H, 11.39. Found: C, 84.81; H, 11.77.

Semicarbazone: Recrystallized from benzene-EtOH, m.p. 203~204° (decomp.). *Anal.* Calcd. for $C_{31}H_{51}ON_3$: C, 77.28; H, 10.67. Found: C, 77.41; H, 10.65.

Oxime: Needles (from $CHCl_3$-MeOH), m.p. 175.5~176.5°. *Anal.* Calcd. for $C_{30}H_{49}ON$: C, 81.94; H, 11.23. Found: C, 81.15; H, 11.36.

Cycloartenyl Ferulate (Oryzanol-A)—A mixture of 0.25 g. of cycloartenol, 0.20 g. of ethoxycarbonylferulic chloride, and 2 cc. of pyridine was allowed to stand for 24 hrs. The product was crystallized from EtOH to 0.30 g. of needles, m.p. 117~119°. *Anal.* Calcd. for $C_{43}H_{62}O_6$: C, 76.52; H, 9.26. Found: C, 76.44; H, 8.96.

A mixture of 0.30 g. of this substance, 10 cc. of benzene, and 30 cc. of 0.1 *N* MeOH-KOH solution was warmed at 50° for 1 hr., neutralized with glacial AcOH, and poured into water. This was extracted with ether and the ether residue was recrystallized from $CHCl_3$-MeOH to 0.20 g. of plates, m.p. 150.5～151.5°, $[\alpha]_D$ +42° (c=0.58). *Anal.* Calcd. for $C_{40}H_{58}O_4 \cdot H_2O$: C, 77.37; H, 9.74. Found : C, 77.31; H, 9.53.

Acetate : m.p. 186～187°, $[\alpha]_D$ +42° (c=0.86). *Anal.* Calcd. for $C_{42}H_{60}O_5$: C, 78.22; H, 9.38. Found : C, 77.95; H, 9.14.

Methyl Ether : m.p. 141～142°, $[\alpha]_D$ +40° (c=0.76). *Anal.* Calcd. for $C_{41}H_{60}O_4$: C, 79.82; H, 9.80. Found : C, 80.30; H, 9.78.

None of these substances showed any depression of the melting point on admixture with oryzanol-A and its acetate and methyl ether, obtained from rice bran oil.

Oryzanol-B Methyl Ether—A mixture of 1.13 g. of oryzanol-B, 1.3 g. of Me_2SO_4, 2.7 g. of K_2CO_3, and 50 cc. of acetone was refluxed for 2.5 hrs. and the product thereof was recrystallized from $CHCl_3$-MeOH to needles, m.p. 149.5～150.5°, $[\alpha]_D$ +51° (c=1.04). *Anal.* Calcd. for $C_{42}H_{62}O_4$: C, 79.95; H, 9.91; CH_3O, 9.84; mol. wt., 630.9. Found : C, 79.78; H, 9.78; CH_3O, 10.62; [illegible] wt., 628.0, 633.

Saponification of Oryzanol-B Methyl Ether—A mixture of 0.681 g. of oryzanol-B methyl ether and 10 cc. of 0.2*N* KOH-EtOH was boiled for 3.5 hrs. The saponified solution consumed 15.79 cc. of 0.05*N* HCl (control : 37.64 cc.). Mol. wt. Found : 628. The reaction mixture was extracted with ether and separated into neutral and acid portions. The acid portion yielded 0.19 g. of 3,4-dimethoxycinnamic acid, m.p. and mixed m.p. 181～182°. *Anal.* Calcd. for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found : C, 63.16; H, 5.95.

The neutral portion was recrystallized from $CHCl_3$-MeOH to 0.40 g. of plates, m.p. 114～115°, $[\alpha]_D$ +55° (c=0.80). Designated as alcohol-B. *Anal.* Calcd. for $C_{31}H_{52}O$: C, 84.48; H, 11.89. Found : C, 84.82; H, 11.56.

Saponification of Oryzanol-B—A mixture of 0.45 g. of oryzanol-B and 40 cc. of 10% KOH-EtOH was boiled for 6 hrs. From the neutral portion, 0.26 g. of alcohol-B, m.p. 114～115°, was obtained and the acid portion yielded 0.12 g. of ferulic acid, m.p. and mixed m.p. 171°.

Alcohol-B Acetate—A mixture of 0.15 g. of alcohol-B, 1 cc. of pyridine, and 1 cc. of Ac_2O was allowed to stand for 24 hrs. The product was recrystallized from $CHCl_3$-MeOH to plates, m.p. 109～110°, $[\alpha]_D$ +68° (c=1.00). I.R. $\nu_{\max}^{CS_2}$ 1645, 887 cm^{-1} ($>C=CH_2$). *Anal.* Calcd. for $C_{33}H_{54}O_2$: C, 82.09; H, 11.27. Found : C, 82.33; H, 10.93.

This acetate (0.1095 g.) was allowed to stand with 10 cc. $CHCl_3$ solution of 0.302 mole of perbenzoic acid in an ice chamber and 1 cc. each was titrated. Consumption of oxygen : 1.25 after 1 day, 1.35 after 2 days, and 1.35 moles after 5 days.

Dibromide : $CHCl_3$ solution of Br_2 was added dropwise into $CHCl_3$ solution of the acetate, under ice cooling, and the residue obtained on evaporation of the solvent was recrystallized from $CHCl_3$-EtOH to plates, m.p. 186～187° (decomp.). Colors pale yellow with tetranitromethane. *Anal.* Calcd. for $C_{33}H_{54}O_2Br_2$: C, 61.69; H, 8.45. Found : C, 62.07; H, 8.26. Debromination with glacial AcOH-Zn regenerated the acetate, m.p. 109～110°.

Alcohol-B Benzoate—A mixture of 0.10 g. of alcohol-B, 1.5 cc. of pyridine, and 0.2 cc. of BzCl was allowed to stand at room temp., and the reaction product was recrystallized from $CHCl_3$-MeOH to plates, m.p. 117.5～118.5°, $[\alpha]_D$ +67° (c=0.76). *Anal.* Calcd. for $C_{38}H_{56}O_2$: C, 83.77; H, 10.36. Found: C, 84.15; H, 10.25.

Alcohol-B *p*-Nitrobenzoate—Prepared with *p*-nitrobenzoyl chloride in pyridine and recrystallized from $CHCl_3$-EtOH to needles, m.p. 227～228°. *Anal.* Calcd. for $C_{38}H_{55}O_4N$: C, 77.37; H, 9.40. Found : C, 77.21; H, 9.36.

Summary

The methyl ether obtained from oryzanol-A and dimethyl sulfate is saponified with ethanolic potassium hydroxide to form 3,4-dimethoxycinnamic acid and a neutral substance. Under a more drastic conditions, oryzanol-A itself is saponified to form the same neutral substance and ferulic acid. The neutral substance is a kind of triterpenoid alcohol and was determined as cycloartenol by the comparison of the constants of various derivatives and a mixed fusion of its acetate and benzoate. Therefore, oryzanol-A is cycloartenyl ferulate and the absorption in the ultraviolet range is due to the ferulic acid moiety.

Oryzanol-B was also found to be the ferulate of a triterpenoid alcohol but the nature of this alcohol was not established. Its properties were similar to cycloartenol but its infrared spectrum revealed the presence of a vinylidene group, while cycloartenol possesses anisopropy lidene group. As shown in Table II, the constants of various derivatives were clearly different from those of cyclolaudenol, which possesses a vinylidene group.

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