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20. Masao Shimizu and Genkichi Ohta : Studies on the Constituents of Rice Bran Oil. V.[1] Reëxamination of Oryzanol-B.

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Along with oryzanol-A and -C, oryzanol-B was isolated from rice-bran oil and was shown to be the ferulate of an alcohol, named tentatively as alcohol-B.[2,3] Originally, oryzanol-B was obtained by deacetylation of oryzanol-B acetate which was separated by fractional recrystallization of oryzanol acetate mixture. It was described in Part III[4] that this method of separation was not satisfactory for oryzanol-B. When oryzanol-A and -C were found to be the ferulates of cycloart-24-enol (I)[3] and 24-methylenecycloartanol (II),[1] respectively, doubt arose whether oryzanol-B is a mixture of oryzanol-A and -C, i.e., alcohol-B is a mixture of (I) and (II). This paper describes the reëxamination and correction of oryzanol-B structure.

Oryzanol-B acetate was previously assumed to be homogeneous but after careful recrystallization from a dilute solution, its melting point rose gradually. The same behavior was observed in the case of alcohol-B acetate. As the attempted separation of oryzanol-B into oryzanol-A and -C was accompanied by a considerable loss of material and did not give a good result, the following experiments were carried out to solve the problem.

Oryzanol acetate mixture, m.p. 202~208°, which contains oryzanol-B acetate,[4] was converted to the corresponding alcohol benzoate by the procedure described previously.[2] The crude benzoate was fractionally recrystallized from acetone-ethyl acetate (1:1) and, from the easily soluble fraction, the seemingly homogeneous alcohol-B benzoate was obtained, which was further converted to alcohol-B and its acetate. Their constants approximated those reported previously, except for the specific rotation of alcohol-B acetate. The previously recorded more positive value ($[\alpha]_D$ +68°) of the acetate was found to be erroneous. A synthetic mixture of compounds of cycloart-24-enol series and the corresponding compounds of 24-methylenecycloartanol series were also prepared. These mixtures (1:1), when crystallized once, showed almost the same melting points as those of corresponding compounds of the alcohol-B series (see Table I). The infrared spectrum of alcohol-B acetate (in CS_2) contained bands corresponding to vinylidene group (1640 and 887 cm^{-1}) and was almost identical with that of 24-methylenecycloartanyl acetate.[4] It was difficult to detect moderately weak bands in 800 cm^{-1} range characteristic to trisubstituted double bond. However, in the far ultraviolet region,[5] absorption of alcohol-B acetate (ε_{206} 2500[*2]) was more intense than that of 24-methylenecycloartanyl acetate (ε_{206} 1530). If alcohol-B acetate contains only a vinylidene group, the intensity of the absorption must be the same as that of 24-methylenecycloartanyl acetate. Cycloart-24-enyl acetate showed far more intense absorption (ε_{206} 3060).

The heterogeneity of alcohol-B was also suggested by these spectral data and finally proved by ozonolysis of the acetate. From the volatile fraction after ozonolysis, formaldehyde and acetone were isolated respectively as their dimedone derivative and 2,4-dinitro-

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*2 Molecular weight of alcohol-B acetate was calculated as $C_{32}H_{52}O_2$.

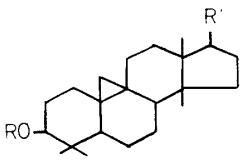
1) Part IV : This Bulletin, 8, 9(1960).
2) M. Shimizu, *et al.* : *Ibid.*, 5, 36(1957).
3) G. Ohta, M. Shimizu : *Ibid.*, 5, 40(1957).
4) Part III : *Ibid.*, 8, 5(1960).
5) cf. P. Bladon, H. B. Henbest, G. W. Wood : J. Chem. Soc., **1952**, 2737.

phenylhydrazone. To obtain non-volatile products, the acetate was ozonized at −60°, reduced with zinc and acetic acid, and then oxidized with chromium trioxide in acetic acid at room temperature. From the reaction mixture, 3β-acetoxy-25,26,27-trisnorcycloartan-24-oic acid[1] (Ⅲ) and 24-oxocycloartanyl acetate[1] (Ⅳ) were isolated. Oxidation of (Ⅳ) with chromium trioxide under the same condition did not give any acid compounds.

In another experiment, oryzanol acetate mixture, m.p. 185~206°,[4] was converted to alcohol-B acetate mixture without purification through the benzoate. Ozonolysis of this alcohol-B acetate mixture also gave the above acetoxy-acid (Ⅲ) and oxo-acetate (Ⅳ) together with a minute amount of cycloartanyl acetate as identified products. From these results it follows that the supposedly homogeneous alcohol-B is largely a mixture of (I) and (Ⅱ), and it seems probable that cycloartanol occurs naturally.

It should be noted here that triterpenes of rice-bran oil, similar to alcohol-B, have already been recorded in the literature and that they are complicated. Karrer, Salomon, and Fritsche[6] described an α-tritisterol which had been obtained from wheat-germ oil. Todd, Bergel, Waldmann, and Work[7] isolated α-, β- and γ-orysterol, and pointed out the similarity between α-tritisterol and γ-orysterol. Both groups of workers mentioned that homogeneity of the sterol was not completely confirmed.

During progress of the present work, two preliminary reports appeared. Yoshida, Takasaki, and Sueyoshi[8] isolated a compound which has properties similar to β-orysterol. They proposed the structure of cycloart-25-enol (V) for it and renamed it β-cycloörysterol. Tsuchiya, Kato, and Endo[9] reported α- and β-oryzayl alcohol obtained by saponification of oryzanol mixture. Comparison of the constants of these compounds



(I) R=H, R'= -CH(Me)-$(CH_2)_2$-CH=C(Me_2)
(Ⅱ) R=H, R'= -CH(Me)-$(CH_2)_2$-C(=CH_2)-CH(Me_2)
(Ⅲ) R=Ac, R'= -CH(Me)-$(CH_2)_2$-COOH
(Ⅳ) R=Ac, R'= -CH(Me)-$(CH_2)_2$-CO-CH(Me_2)
(V) R=H, R'= -CH(Me)-$(CH_2)_3$-C$\langle^{CH_2}_{Me}$

Table I.

	Alcohol		Acetate		Benzoate		*p*-Nitrobenzoate
	m.p. (°C)	$[\alpha]_D$	m.p. (°C)	$[\alpha]_D$[a]	m.p. (°C)	$[\alpha]_D$[a]	m.p. (°C)
Cycloart-24-enol (I)	114~115	+52°[a]	122~124	+59°	129~130	+75°	217
24-Methylenecycloartanol (Ⅱ)	121~122	+43°[a]	116~117	+54°	156~157	+62°	235
Synthetic mixture of (I) and (Ⅱ)	113~115		107~110	+57°	117~119		225~227
Alcohol-B	113~115	+50°[a]	108~110	+58°	116~117	+70°	226~227
α-Tritisterol	114~115	+54.3°[b]	107~108	+70°			
α-Orysterol	121~122	+49°[b]					187
β-Orysterol	113~114	+51.3°[b]	104				227~228
γ-Orysterol	119~120	+51.9°[b]					233~234
α-Oryzayl alcohol	155.8~156.7						
β-Oryzayl alcohol	110~110.7						

a) Taken in $CHCl_3$. *b*) in EtOH.

6) P. Karrer, H. Salomon, H. Fritsche: Helv. Chim. Acta, **20**, 1422(1937). cf. P. Karrer, H. Salomon: *Ibid.*, **20**, 424(1937).
7) A. R. Todd, F. Bergel, H. Waldmann, T. S. Work: Biochem. J., **31**, 2247(1937).
8) S. Yoshida, R. Takasaki, H. Sueyoshi: Yakugaku Zasshi, **76**, 1333(1956).
9) T. Tsuchiya, A. Kato, T. Endo: Tokyo Kogyo Shikensho Hokoku, **51**, 359(1956)(C. A., **51**, 4320 (1957)); cf. T. Tsuchiya, A. Kato: *Ibid.*, **53**, 230(1958).

given in Table I shows that γ-orysterol is similar to 24-methylenecycloartanol and that α-tritisterol and β-orysterol are similar to alcohol-B. Despite the communication of Yoshida, *et al.*, it seems probable that β-orysterol is a mixture like alcohol-B.

Experimental

(All m.p.s are uncorrected, $[\alpha]_D$ refers to $CHCl_3$, and ultraviolet absorptions were measured in EtOH solution with Hitachi E. P. S-II Spectrophotometer)

Synthetic Mixture of Oryzanol-A[2] and -C,[4] and their Derivatives—A mixture of oryzanol-A (m.p. 148~150°)(10 mg.) and oryzanol-C (m.p. 162~164°/193~194°)(10 mg.) was crystallized once from $CHCl_3$-MeOH to plates, m.p. 144~147° (reported[2] for oryzanol-B, m.p. 150°). One crystallization of the mixture of oryzanol-A acetate (m.p. 186~187°)(10 mg.) and oryzanol-C acetate (m.p. 216°)(10 mg.) from Me_2CO gave small needles of m.p. 199~204° (reported[2] for oryzanol-B acetate, m.p. 204~206°). For other mixtures see Table I.

Alcohol-B Acetate from Oryzanol-B Acetate Mixture—a) Oryzanol-B acetate mixture, m.p. 202~208°,[4] was deacetylated and then saponified by the procedure reported previously.[2] The neutral product (6.9 g.) was benzoylated with BzCl and pyridine. The crude benzoate (m.p. 114°, cloudy to 125°) was fractionally recrystallized from Me_2CO-AcOEt (1:1). The less soluble compounds melting in the range of 117~145° (3.8 g.) were discarded. From the mother liquor, plates of m.p. 110~116° were obtained. Three crystallizations from Me_2CO gave alcohol-B benzoate as plates (1.9 g.), m.p. 116~117°; $[\alpha]_D$ +70° (c=1.53) (reported[3] m.p. 117.5~119.5°; $[\alpha]_D$ +68°). *Anal.* Calcd. for $C_{37}H_{54}O_2$: C, 83.72; H, 10.25. Calcd. for $C_{38}H_{56}O_2$: C, 83.77; H, 10.36. Found: C, 83.70; H, 10.53.

Hydrolysis of the benzoate, followed by one crystallization from MeOH gave plates, m.p. 111~114°; $[\alpha]_D$ +47° (c=1.87). Two more recrystallizations gave alcohol-B of m.p. 113~115°, $[\alpha]_D$ +50° (c=3.83) (reported[3] m.p. 114~115°, $[\alpha]_D$ +55°).

Acetylation of alcohol-B and crystallization of the product once from $CHCl_3$-MeOH gave alcohol-B acetate as blades, m.p. 108~110°, $[\alpha]_D$ +57° (c=4.34). Three more crystallizations gave blades of m.p. 108~110° (cloudy to 112°), $[\alpha]_D$ +58° (c=2.42). *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Calcd. for $C_{33}H_{54}O_2$: C, 82.09; H, 11.27. Found: C, 81.70; H, 11.24. IR $\gamma_{max}^{CS_2}$ cm^{-1}: 3040, 1640, 1242, 1039. 1023, 977, 887. UV: ε_{260} 2500, ε_{210} 1860.*2

(For comparison: Cycloart-24-enyl acetate, ε_{206} 3060, ε_{210} 2300. 24-Methylenecycloartany lacetate, ε_{206} 1530, ε_{210} 740). Reëxamination of the earlier samples of alcohol-B acetate (reported[3] m.p. 109~110°, $[\alpha]_D$ +68°) showed $[\alpha]_D$ +56° (c=0.77).

A mixture of cycloart-24-enyl acetate (50 mg.) and 24-methylenecycloartanyl acetate (50 mg.) was crystallized once from $CHCl_3$-MeOH to blades (65 mg.) of m.p. 107~110°, $[\alpha]_D$ +57° (c=1.92). UV: ε_{207} 1950, ε_{210} 1360. The IR curve was almost identical with that of alcohol-B acetate.

b) Oryzanol-B acetate mixture[4] of m.p. 180~206° was converted to alcohol-B acetate mixture without purification through the benzoate. The acetate crystallized once from $CHCl_3$-MeOH (m.p. 104~110°) and used for ozonolysis (v. i.).

Alcohol-B *p*-Nitrobenzoate: Prepared from alcohol-B with *p*-nitrobenzoyl chloride and pyridine, and separated from Me_2CO as blades, m.p. 226~227°. *Anal.* Found: C, 77.45; H, 9.87.

Ozonolysis of Alcohol-B Acetate—a) Alcohol-B acetate (0.86 g.) was dissolved in AcOH (40 cc.) and ozonized oxygen (1.2 moles of O_3) was passed through at room temperature. The gas evolved was collected in a trap of ice-cold water (40 cc.). The acetic acid and water were combined, 10% $FeSO_4$ solution (20 cc.) was added, and the mixture was steam-distilled into ice-cold water (50 cc.). To the neutralized distillate (300 cc.), 1% dimedone solution (25 cc.) was added and left to stand overnight. Separated crystals (70 mg., m.p. 185~189°) were collected and crystallized from hydr. MeOH to formaldehyde dimethone as needles, m.p. and mixed m.p. 189°. *Anal.* Calcd. for $C_{17}H_{24}O_4$: C, 69.83; H, 8.29. Found: C, 69.98; H, 8.15.

The filtrate left after removal of dimedone derivative was again steam distilled into water. Treatment of the distillate (300 cc.) in the usual way with 2,4-dinitrophenylhydrazine hydrochloride gave a yellow precipitate (0.11 g., m.p. 120~125°). After chromatography in benzene solution over alumina, this furnished acetone 2,4-dinitrophenylhydrazone (recrystallized from MeOH), m.p. 124~125°, undepressed on admixture with an authentic specimen of m.p. 125~126°. *Anal.* Calcd. for $C_9H_{10}O_4N_4$: C, 45.38; H, 4.25; N, 23.52. Found: C, 45.82; H, 4.08; N, 23.92.

b) Alcohol-B acetate (2.00 g.) was dissolved in purified $CHCl_3$ (100 cc.) and treated with ozonized oxygen (1.2 moles of O_3) at −60°. The solution was treated with AcOH (20 cc.) and zinc dust (12 g.), and stirred at room temperature for 4.5 hr. The filtered solution was washed with water, dried, and evaporated. The residue was dissolved in AcOH (20 cc.) to which an aqueous solution of CrO_3 (0.2 g. in

2 cc.) was added and kept at room temperature over night. After dilution with water, the mixture was shaken with Et_2O. The ether solution was washed with water and 5% K_2CO_3 solution, the solid that separated at the interface was run off with the aqueous layer, which was acidified with dil. HCl and extracted with $CHCl_3$. The residue (0.80 g.) on evaporation of $CHCl_3$ solution was crystallized from Me_2CO to 0.43 g. of 3β-acetoxy-25,26,27-trisnorcycloartan-24-oic acid[1] as plates, m.p. and mixed m.p. 216~217°; $[\alpha]_D$ +58° (c=2.20). *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.62; H, 10.03. Identity was also confirmed by comparison of the IR curves.

With ethereal CH_2N_2, the acid gave the methyl ester acetate, m.p. 121~122°, undepressed on admixture with authentic methyl 3β-acetoxy-25,26,27-trisnorcycloartan-24-oate, $[\alpha]_D$ +56° (c=2.00). *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.21; H, 9.98.

The ether solution was evaporated, the residue (1.05 g.) was dissolved in light petroleum (100 cc.), percolated through alumina (45 g.), and eluted with (a) light petroleum (600 cc.) and (b) light petroleum-benzene (9:1, 350 cc., 4:1, 200 cc. and 1:1, 200 cc.). The evaporated residue of fraction (b) (0.80 g.) was crystallized from $CHCl_3$-MeOH to 0.61 g. of blades, m.p. 121~122°, alone or mixed with 24-oxocycloartanyl acetate.[1] $[\alpha]_D$ +52° (c=1.63). *Anal.* Calcd. for $C_{32}H_{52}O_3$: C, 79.28; H, 10.81. Found: C, 79.51; H, 11.09. Identity was also confirmed by comparison of IR curves.

The oxime separated as needles from Et_2O-light petroleum, m.p. 192~194°, undepressed on admixture with an authentic specimen of the same m.p. $[\alpha]_D$ +48° (c=2.27). *Anal.* Calcd. for $C_{32}H_{53}O_3N$: C, 76.90; H, 10.69. Found: C, 77.09; H, 10.56.

Treatment of 24-oxocycloartanyl acetate (70 mg.) in AcOH (3 cc.) with CrO_3 (30 mg.) in water (0.3 cc.) for 12 hr. at room temperature did not give any acid product. A neutral product furnished the unchanged starting material, separating from aqueous MeOH as blades (45 mg.), m.p. and mixed m.p. 121~122°.

c) Alcohol-B acetate mixture (m.p. 104~110°) (8.2 g.) was ozonized, reduced, and oxidized, and the product was separated into acid and neutral portions as described in b). From the acid portion (2.4 g.), 3β-acetoxy-25,26,27-trisnorcycloartan-24-oic acid (1.55 g.) was isolated, m.p. and mixed m.p. 217~219°. $[\alpha]_D$ +58° (c=2.41). The neutral portion (5.2 g.) was dissolved in light petroleum (100 cc.), chromatographed through alumina (150 g.), and eluted with (a) light petroleum (1.2 L.), (b) light petroleum (500 cc.), (c) light petroleum (800 cc.) and light petroleum-benzene (9:1, 1.5 L.), (d) light petroleum-benzene (9:1, 600 cc.), and (e) light petroleum-benzene (9:1, 1 L. and 1:1, 2.5 L.). Fraction (a) gave a semi-solid (80 mg.), and a product of m.p. 120~129° (0.12 g.) was obtained from fraction (b), which was crystallized from $CHCl_3$-MeOH to give cycloartanyl acetate as needles (80 mg.), m.p. and mixed m.p. 131~132°. $[\alpha]_D$ +58° (c=0.77). UV ε_{206} 500. Its IR spectrum was identical with that of authentic specimen.[1] *Anal.* Calcd. for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56. Found: C, 81.20; H, 11.08.

Hydrolysis of the acetate gave cycloartanol, plates (from MeOH), m.p. and mixed m.p. 99~101°. Analytical sample, dried *in vacuo*, melted at 100° (cloudy to 106°); $[\alpha]_D$ +48° (c=0.94). *Anal.* Calcd. for $C_{30}H_{52}O \cdot \frac{1}{2} CH_3OH$: C, 82.35; H, 12.25. Found: C, 82.55; H, 11.97.

Fraction (c) gave a product (0.46 g.) which on crystallization from $CHCl_3$-MeOH afforded blades of m.p. 102~106°. The IR curve suggested that this is the unreacted starting material. IR: $\gamma_{max}^{CS_2}$ 1640, 887 cm^{-1}.

Fraction (d) gave a product of m.p. 113~120° (cloudy to 135°) (0.28 g.), which contained a minute amount of a product of higher melting point and has not been examined in detail.

From fraction (e) a product of m.p. 117~120° (3.48 g.) was obtained, which was crystallized from $CHCl_3$-MeOH to 24-oxocycloartanyl acetate as blades, m.p. and mixed m.p. 121~123°, $[\alpha]_D$ +54° (c=1.96) (2.09 g.).

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Summary

Oryzanol-B was shown to be largely a mixture of cycloart-24-enyl ferulate (oryzanol-A) and 24-methylenecycloartanyl ferulate (oryzanol-C) by comparison of melting points of their derivatives and by degradation. Alcohol-B, obtained by saponification of oryzanol-B, was acetylated and ozonolysis of this acetate afforded 24-oxocycloartanyl acetate and 3β-acetoxy-25,26,27-trisnorcycloartan-24-oic acid. Comparison of alcohol-B with reported triterpenes of rice-bran oil is described.

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