UDC 547.913:633.18

2. Genkichi Ohta : Studies on the Constituents of Rice-Bran Oil. III.[1] Structure of Oryzanol-C.[2] (1).

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The isolation of three phenolic substances, oryzanol-A, -B and -C, from rice-bran oil was reported previously.[1,3] Oryzanol-A was identified as cycloartenyl ferulate and oryzanol-B was shown to be the ferulate of a triterpenoid alcohol, designated tentatively as alcohol-B, whose structure was assumed to be similar to cycloartenol. Oryzanol-C was characterized only by its acetate. Previous experiments showed that acetylation of the crude oryzanol, followed by fractional recrystallization could separate the three oryzanol acetates. During the progress of this study it was observed that oryzanol-A is less soluble than oryzanol-B and -C in common organic solvents while among the acetyl derivatives oryzanol-C acetate is the most insoluble. Consequently, oryzanol-A or oryzanol-C acetate can be obtained in the homogeneous state, but there is some doubt as to the homogenity of oryzanol-B. Consequently, oryzanol-C was examined prior to oryzanol-B.

The isolating procedure was modified from the previous experiments for simplification. A solution of crude rice-bran oil in dichloroethane was deacidified with sodium carbonate solution and then extracted with potassium hydroxide solution, from which a phenolic oil was obtained in 1.5% yield. The phenolic oil was treated with methanol to separate a solid. The solid was adsorbed on silica gel and eluted successively with methanol, ethanol, and acetone. The earlier fraction which colored blue-green in the Liebermann-Burchard reaction has not been examined in detail. The later fraction was acetylated, fractionally recrystallized from ethyl acetate, and divided into four fractions; m.p. 204~212°, m.p. 187~206°, m.p. 175~185°, and m.p. 155~170°. The acetate of m.p. 155~170° gave a blue-green color in the Liebermann-Burchard reaction and as yet has not been examined further. Deacetylation of the acetate of m.p. 175~185°, followed by repeated crystallization gave oryzanol-A. The acetate of m.p. 187~206° contains oryzanol-B acetate about which it is hoped to report elsewhere. The sparingly soluble acetate of m.p. 204~212° was again adsorbed on silica gel and eluted with ethyl acetate. From the later fraction, oryzanol-C acetate, m.p. 216~218°, $[\alpha]_D$ +39° (in $CHCl_3$), was obtained in an improved yield (0.03%) and deacetylated under weakly alkaline conditions to oryzanol-C, m.p. 162~164°/193~194°, $[\alpha]_D$ +36° (in $CHCl_3$), which was further characterized as its methyl ether.

Oryzanol-C, as well as oryzanol-A and -B, gave a red to yellow color in the Liebermann-Burchard reaction and showed an absorption maximum at 328 mμ[*2] in the ultraviolet spectra indicating the presence of the ferulate moiety. Drastic saponification of oryzanol-C gave ferulic acid and a neutral substance designated for convenience as alcohol-C. Saponification of oryzanol-C methyl ether proceeded smoothly to give 3,4-dimethoxycinnamic acid and alcohol-C. The molecular formula of oryzanol-C appears to be $C_{41}H_{60}O_4$ from the saponification equivalent of the methyl ether and from

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*2 The UV spectra were taken by Hitachi E. P. S. II self-recording spectrophotometer in EtOH solution. In earlier experiments Hitachi M. P. B-U. spectrophotometer was used and the ferulic acid ester showed maximum absorption at 322 mμ.[1,3]

1) Part II. This Bulletin, **5**, 40(1957).
2) Preliminary accounts were published as a Communication to the Editor in this Bulletin, **6**, 325 (1958).
3) M. Shimizu, *et al.* : *Ibid.*, **5**, 36(1957).

analytical values, and so formulation as $C_{31}H_{52}O$ is favored for alcohol-C.

The alcoholic function of alcohol-C was characterized by formation of an acetate and a benzoate, and by Oppenauer oxidation to a ketone. Titration of alcohol-C acetate with perbenzoic acid revealed the presence of one double bond, and an epoxy acetate was obtained as the reaction product. Catalytic hydrogenation of alcohol-C or its acetate with platinum catalyst afforded the corresponding dihydric compound. Alcohol-C acetate was brominated to its dibromide. The ultraviolet absorption of alcohol-C acetate suggested the double bond to be disubstituted[4] and absorptions at 1639 and 887 cm^{-1} in its infrared spectrum indicated the presence of a vinylidene group.

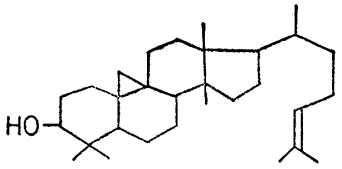
A close relationship between alcohol-C and cycloartenol (I)[5,6] was suggested by detailed examination of the infrared spectra of alcohol-C and its acetate, although it was difficult to resolve sufficiently bands in the 3000 cm^{-1} range owing to the use of sodium chloride prism. Alcohol-C exhibited absorptions at 1045, 1020, 1005, and 988 cm^{-1}, and its acetate at 1242, 1039, 1022, and 978 cm^{-1} (in CS_2). These two groups of absorptions are identical with those found by Allsop, Cole, White, and Willix[7] for 3β-hydroxyl and 3β-acetoxyl group of cycloartenol and its acetate, respectively. A band near 3040 cm^{-1} in the spectra of alcohol-C derivatives also suggests the presence of cyclopropane ring.[8] It was therefore assumed that alcohol-C has the same ring system as cycloartenol, and other observations support this view. First, in contrast to their saturated nature, dihydro-alcohol-C, dibromo-alcohol-C acetate, and epoxy-alcohol-C acetate gave a positive reaction with tetranitromethane, attributable to the presence of a cyclopropane ring.[5] Second, the change in molecular rotation accompanying oxidation of alcohol-C to the corresponding ketone was strongly negative (ΔM −101°)*3 and roughly equal to those observed for cycloartenol[5] and cyclolaudenol.[9] It has been already shown by Henry, Irvine, and Spring[10] that cyclolaudenol, the C_{31}-triterpene containinga cyclopropane ring and a vinylidene group, is represented as (II). The physical constants given in Table I show that alcohol-C is not identical with cyclolaudenol, e.g. there is a remarkable difference between their benzoyl derivatives. As will be reported in the following paper, alcohol-C is 24-methylenecycloartanol and differs in the position of the double bond from cyclolaudenol. Alcohol-B,[3] obtained by saponification of oryzanol-B, seems to have similar properties, and it is hoped to report later on alcohol-B and other related compounds.

Table I.

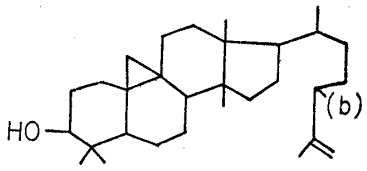
	m.p. (°C)	$[\alpha]_D$ ($CHCl_3$)		m.p. (°C)	$[\alpha]_D$ ($CHCl_3$)
Alcohol-C	121~122	+43°	Cyclolaudenol	125	+46°
Ketone	111~112	+20°	Cyclolaudenone	115	+19°
Alcohol-C acetate	116~117	+54°	Cyclolaudenyl acetate	120~121	+55°
Alcohol-C benzoate	156~157	+62°	Cyclolaudenyl benzoate	194~195	+63°
Dihydro-alcohol-C	135~136	+46.5°	Cyclolaudanol	133~134	+43°
Dihydro-alcohol-C acetate	123~124	+54.5°	Cyclolaudanyl acetate	132~133	+50°

*3 In most triterpenes,[5] including cycloeucalenol,[11] this change of molecular rotation is positive. For the negative change, cf. W. Lawrie, W. H. Hamilton, F. S. Spring, H. S. Watson: J. Chem. Soc., **1956**, 3274.

4) cf. P. Bladon, H. B. Henbest, G. W. Wood: J. Chem. Soc., **1952**, 2732.
5) D. H. R. Barton: *Ibid.*, **1951**, 1444.
6) D. S. Irvine, J. A. Henry, F. S. Spring: *Ibid.*, **1955**, 1317.
7) I. L. Allsop, *et al.*: *Ibid.*, **1956**, 4868. According to their report cycloartanol has a band at 1012, instead of at 1004 cm^{-1}. In the present experiments cycloartanol showed the identical bands in this range as those of cycloartenol.
8) A. R. H. Cole: J. Chem. Soc., **1954**, 3810
9) H. R. Bentley, J. A. Henry, D. S. Irvine, D. Mukerji, F. S. Spring: *Ibid.*, **1955**, 596.
10) J. A. Henry, D. S. Irvine, F. S. Spring: *Ibid.*, **1955**, 1607.
11) J. S. G. Cox, F. E. King, T. J. King: *Ibid.*, **1956**, 1384.



(I)



(II)

Experimental

(All m.p.s are uncorrected, $[\alpha]_D$ taken in $CHCl_3$. Ultraviolet spectra*[2] were measured in EtOH solution and infrared spectra were measured by Hitachi E. P. I. 2 spectrophotometer)

Isolation of Oryzanol-C—A solution of rice-bran oil in dichloroethane was deacidified with 40% Na_2CO_3 solution and then extracted with KOH solution, neutralisation of which gave a phenolic oil (740 g. from 48 kg. of rice-bran oil). The phenolic oil was triturated with MeOH to separate a solid (170 g.). The solid was dissolved in $CHCl_3$ (500 cc.) and silica gel (500 g., 100 mesh) was added to it. The mixture was evaporated to dryness, and the residue, placed on the bed of silica gel (2 kg.), was eluted with (a) MeOH (13 L.) and MeOH-EtOH (1:1, 13 L.), and (b) EtOH (15 L.), EtOH-acetone (4:1, 12 L. and 1:1, 12 L.), and acetone (12 L.). After evaporation of solution (a) an oily product (34 g.) was obtained. The evaporated residue (120 g.) of solution (b) was acetylated with pyridine (220 cc.) and Ac_2O (120 cc.), and the product was fractionally recrystallized from AcOEt and divided into four fractions in the order of increasing solubility as follows: (c) m.p. 204~212° (29 g.), (d) m.p. 187~206° (32 g.), (e) m.p. 175~185° (30 g.), and (f) m.p. 155~170° (16 g.) (m.p. is the range in which compounds melted). Fraction (c) was mixed with silica gel (200 g.) in $CHCl_3$ as above, placed on the column of silica gel (750 g.), and eluted with AcOEt. Total of 21 fractions of 200 cc. each were obtained. The evaporated residue of fractions (8~20) was combined and recrystallized from AcOEt-benzene to oryzanol-C acetate (15 g.) as blades, m.p. 216~218°; $[\alpha]_D$ +39° (c=2.71). *Anal.* Calcd. for $C_{43}H_{62}O_5$: C, 78.38; H, 9.48. Found: C, 78.26, 78.37; H, 9.80, 9.17.

To a solution of the acetate (15 g.) in benzene (50 cc.), 0.2*N* methanolic KOH solution (150 cc.) was added and kept at 50° for 1 hr. After neutralisation with AcOH, the mixture was concentrated *in vacuo*, water was added, the product was isolated by means of ether, and crystallized from $CHCl_3$-MeOH. Oryzanol-C separated as plates (13.5 g.), m.p. 162~164°/193~194°; $[\alpha]_D$ +36° (c=1.87). UV λ_{max} 328 mμ (log ε 4.29). *Anal.* Calcd. for $C_{41}H_{60}O_4$: C, 79.82; H, 9.80. Found: C, 79.59; H, 10.07.

Acetylation of oryzanol-C regenerated oryzanol-C acetate, m.p. and mixed m.p. 216~218°; $[\alpha]_D$ +40° (c=1.25).

Fraction (d) contained oryzanol-B acetate. Deacetylation of fraction (e), followed by repeated recrystallization from EtOH-benzene gave oryzanol-A[3] (10 g.), m.p. 120° (sint.) to 150°; $[\alpha]_D$ +40° (c=1.00).

The acetate of oryzanol-A, m.p. 186~187°; $[\alpha]_D$ +40° (c=2.98). Fractions (a) and (f) gave blue-green color in the Liebermann-Burchard reaction.

Oryzanol-C Methyl Ether—A mixture of oryzanol-C (1.40 g.), K_2CO_3 (2.76 g.), Me_2SO_4 (1.26 g.), and acetone (50 cc.) was heated under reflux for 2.5 hr. The product, isolated in the usual manner, was crystallized from $CHCl_3$-MeOH to afford the methyl ether as blades (1.05 g.), m.p. 156~157°; $[\alpha]_D$ +38.5° (c=2.26). *Anal.* Calcd. for $C_{42}H_{62}O_4$: C, 79.95; H, 9.91. Found: C, 80.01; H, 9.78.

Alcohol-C—A solution of oryzanol-C (9.8 g.) in 10% ethanolic KOH solution (700 cc.) was refluxed for 6 hr., and the product was separated into neutral and acid portions. Crystallization of the acid portion from hydr. EtOH gave ferulic acid (2.3 g.), m.p. and mixed m.p. 171°. *Anal.* Calcd. for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.40; H, 5.40.

The neutral portion was crystallized from EtOH-MeOH (1:4) to alcohol-C (6.0 g.), as blades of m.p. 121~122°, $[\alpha]_D$ +43° (c=1.97). $[M]_D$ +189°. UV $\varepsilon^{max}_{206m\mu}$ 1440, $\varepsilon_{210m\mu}$ 680. IR cm^{-1} (in CS_2): 3600, 3070, 3039, 1639, 1045, 1020, 1005, 988, 887. *Anal.* Calcd. for $C_{31}H_{52}O$: C, 84.48; H, 11.89. Found: C, 84.30; H, 11.66.

Saponification of oryzanol-C methyl ether (see below) gave alcohol-C, together with 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, 63.50; H, 5.93.

Alcohol-C Acetate: Prepared from alcohol-C with pyridine and Ac_2O at room temperature, and separated from $CHCl_3$-MeOH as blades, m.p. 100~116°, raised after drying *in vacuo*, to 116~117°. $[\alpha]_D$ +54° (c=2.75). UV $\varepsilon^{206m\mu}_{max}$ 1530, $\varepsilon_{210m\mu}$ 740. IR (CS_2) cm^{-1}: 3080, 3040, 1639, 1242, 1039, 1022, 978,

887. *Anal.* Calcd. for $C_{33}H_{54}O_2$: C, 82.09; H, 11.27. Found : C, 82.48; H, 11.06.

Deacetylation of the acetate with 5% methanolic KOH solution gave alcohol-C, m.p. and mixed m.p. 121~122°, $[\alpha]_D$ +43°(c=1.34).

Alcohol-C Benzoate : Prepared by benzoylation of alcohol-C with pyridine and BzCl at 100°. Prismatic needles (from $CHCl_3$-MeOH), m.p. 156~157°; $[\alpha]_D$ +62°(c=2.16). *Anal.* Calcd for $C_{38}H_{56}O_2$: C, 83.77; H, 10.36. Found : C, 83.79; H, 10.49.

Hydrolysis of the benzoate with 5% methanolic KOH solution gave alcohol-C, m.p. 121°, $[\alpha]_D$ +43°(c=1.23).

Alcohol-C *p*-Nitrobenzoate : Prepared in analogous way. Blades (from $CHCl_3$-MeOH), m.p. 234~235°. *Anal.* Calcd for $C_{38}H_{55}O_4N$: C, 77.37; H, 9.40. Found : C, 77.37; H, 9.38.

Alcohol-C Ferulate (Oryzanol-C)—Alcohol-C (0.20 g.) in pyridine solution (4 cc.) was heated at 100° with ethoxycarbonylferulyl chloride (0.20 g.) for 1 hr. The product was crystallized from $CHCl_3$-MeOH in needles of m.p. 149~150° (0.27 g.). *Anal.* Calcd. for $C_{44}H_{64}O_6$: C, 76.70; H, 9.36. Found: C, 77.07; H, 9.37.

Hydrolysis of this compound (0.25 g.) with 0.1*N* methanolic KOH solution (20 cc.) for 1 hr. at 50° gave the ferulate, m.p. and mixed m.p. 162~164°/193~194°, $[\alpha]_D$ +35°(c=1.25). Acetate : m.p. and mixed m.p. 216~218°, $[\alpha]_D$ +38°(c=1.16). *Anal.* Calcd. for $C_{43}H_{62}O_5$: C, 78.38; H, 9.48. Found : C, 78.18; H, 9.20.

Oppenauer Oxidation of Alcohol-C—A mixture of alcohol-C (0.21 g.), cyclohexanone (1 cc.), and toluene (20 cc.) was heated, 10 cc. of the solvent was distilled off, Al(*tert*-BuO)$_3$ (0.3 g.) was added, and the mixture was refluxed for 6 hr. After destroying the Al-compound with 10% KOH solution, ether was added. The ether layer was separated, washed with water, dried, and evaporated to dryness *in vacuo*. Purification of the product by chromatography over alumina and crystallization from $CHCl_3$-MeOH gave the corresponding ketone as blades (0.135 g.), m.p. 111~112°; $[\alpha]_D$ +20°(c=1.29). $[M]_D$ +88°. *Anal.* Calcd. for $C_{31}H_{50}O$: C, 84.86; H, 11.49. Found : C, 84.40; H, 11.66.

Oxime : Needles (from $CHCl_3$-MeOH), m.p. 189~190°. *Anal.* Calcd. for $C_{31}H_{51}ON$: C, 82.06; H, 11.33. Found : C, 82.06; H, 11.28.

Alcohol-C Acetate Dibromide—To a solution of alcohol-C acetate (0.40 g.) in CCl_4 (10 cc.), was added a solution of bromine in the same solvent (8 cc., Br 16 mg./cc.) at 0°. Crystallization of the product from $CHCl_3$-MeOH gave the dibromide as plates (0.25 g.), m.p. 185~186° (decomp. by rapid-heating); $[\alpha]_D$ +37° (c=2.78). *Anal.* Calcd. for $C_{33}H_{54}O_2Br_2$: C, 61.69; H, 8.47. Found : C, 62.41; H, 8.21.

Epoxy-alcohol-C Acetate—Alcohol-C acetate(0.5138 g.) and 20 cc. of 0.171*N* solution of perbenzoic acid in $CHCl_3$ were allowed to react for 21 hr. at 0° and 0.95 atom of oxygen, as determined iodometrically, was absorbed. The solution was washed with 5% Na_2CO_3 solution and water, and dried. The solvent was removed *in vacuo*, the residue was dissolved in light petroleum, and chromatographed through alumina. The fractions eluted with light petroleum and light petroleum-benzene (9:1) gave crystals of m.p. 130~135° (0.22 g.), which was recrystallized from $CHCl_3$-MeOH to afford the epoxy acetate as needles, m.p. 133~135°; $[\alpha]_D$ +55°(c=1.55). *Anal.* Calcd. for $C_{33}H_{54}O_3$: C, 79.46; H, 10.92. Found : C, 79.35; H, 10.53.

Dihydro-alcohol-C—Alcohol-C (0.60 g.) in AcOH (100 cc.) was hydrogenated using a Pt catalyst (from 0.15 g. of PtO_2) for 30 min. Working up in the usual way and recrystallization from $CHCl_3$-MeOH gave dihydroalcohol-C as plates (0.48 g.), m.p. 135.5~136.5°; $[\alpha]_D$ +46.5°(c=1.25); $[M]_D$ +205°. IR cm^{-1} (CS_2) : 3600, 3040, 1045, 1020, 1005, 988. The spectrum lacked bands at 3070, 1639, and 887 cm^{-1}. *Anal.* Calcd. for $C_{31}H_{54}O$: C, 84.09; H, 12.29. Found : C, 84.22; H, 12.18.

Acetylation of dihydroalcohol-C or hydrogenation of alcohol-C acetate gave dihydro-alcohol-C acetate as blades (from $CHCl_3$-MeOH), m.p. 123~124°; $[\alpha]_D$ +54.5°(c=1.58), +55°(c=1.66); $[M]_D$ +264. UV $\varepsilon_{206m\mu}$ 470, $\varepsilon_{210m\mu}$ 280. IR (in CS_2). cm^{-1} : 3040, 1240, 1039, 1024, 978. *Anal.* Calcd. for $C_{33}H_{56}O_2$: C, 81.75; H, 11.64. Found : C, 81.60: H, 11.64.

Saponification Equivalent[12]—The esters were saponified with 0.2*N* ethanolic KOH solution (10 cc.) for 4 hr. at refluxing temperature and titrated with 0.05*N* HCl (phenolphthalein). The results are given in Table II.

Table II.

Substance	(g.)	Equivalent 0.05*N* HCl (cc.)	Mol. formula	Mol. wt. Found	Mol. wt. Calcd.
Oryzanol-C methyl ether	0.6041	19.28	$C_{42}H_{62}O_4$	626.7	630.9
〃	0.3968	12.67	〃	626.4	630.9
Alcohol-C acetate	0.4131	16.98	$C_{33}H_{54}O_2$	486.6	432.7

12) cf. H. Sandquist, J. Gorton : Ber., **63**, 1935(1930).

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Summary

A phenolic compound, oryzanol-C, isolated from rice-bran oil, was shown to be a ferulate of an alcohol, tentatively named alcohol-C. Some derivatives of alcohol-C were prepared. Alcohol-C is a new triterpene of C_{31}-skeleton. Examination of infrared spectra and other chemical properties indicates that alcohol-C contains a vinylidene group and the same ring system as that of cycloartenol (I). Alcohol-C is not identical with cyclolaudenol (II).

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3. Genkichi Ohta: Studies on the Constituents of Rice-Bran Oil. IV.[1] Structure of Oryzanol-C. (2).

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In the preceding paper,[1] oryzanol-C obtained from rice-bran oil, was shown to be the ferulate of an alcohol, designated as alcohol-C. It was observed that alcohol-C is a C_{31}-triterpene containing a vinylidene group and same ring system as cycloartenol (I),[2] and not identical with cyclolaudenol (II).[3] The structure of alcohol-C was elucidated as 24-methylenecycloartanol (III) by the following experiments.

Ozonolysis of alcohol-C acetate afforded a ketone, demethyloxo-alcohol-C acetate (IV) and formaldehyde, isolated in good yield as its dimedone derivative. Thus, in agreement with the spectral data,[1] the presence of a vinylidene group was confirmed. Demethyl-oxo-alcohol-C acetate gave a positive Zimmermann test, showed bands at 1420 and 1710 cm^{-1} (in Nujol) in the infrared spectrum indicating the presence of $-COCH_2-$, and was different from oxonorcyclolaudanyl acetate (V),[4] derived from cyclolaudenyl acetate by ozonolysis. Demethyl-oxo-alcohol-C acetate was stable to treatment with alkali, apart from hydrolysis of the acetate group, which indicates the absence of asymmetric carbon atom adjacent to the carbonyl group. On the contrary, oxonorcyclolaudanyl acetate has been shown[3] to be converted by the same treatment to oxonorcyclo-24ab-laudanol (VI), a difficultly separable mixture of 24a- and 24b-epimers. Although demethyl-oxo-alcohol-C acetate showed muchthe same melting point and optical rotation as those of oxonorcyclo-24ab-laudanyl acetate, the constants of their derivatives were quite different (see Experimental), excluding the possibility that alcohol-C is a mixture of cyclolaudenol and its 24a-epimer. Wolff-Kishner reduction of demethyl-oxo-alcohol-C acetate, followed by acetylation, gave demethyl-alcohol-C acetate which was identified

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1) Part III. This Bulletin, 8, 5(1960).
2) cf. D. S. Irvine, J. A. Henry, F. S. Spring: J. Chem. Soc., **1955**, 1317.
3) cf. *Idem.*: *Ibid.*, **1955**, 1607.
4) H. R. Bentley, *et al.*: *Ibid.*, **1955**, 596.