The writer expresses his deep gratitude to Dr. Junzo Shinoda, President of this Company, to Mr. Sakan Hashimoto, Director of this Laboratory, for kind encouragement, and to Dr. Masao Shimizu, Acting Director, for kind guidances. The writer is also indebted to Mr. S. Kitahara for technical help and to Messrs. B. Kurihara and K. Abe for elemental analyses.

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.

as cycloartanyl acetate (VII). The C_{31}-skeleton and the ring structure of alcohol-C was thus proved.

The location of the ethylenic linkage was shown to be between C-24 and C-28 as follows : Demethyl-oxo-alcohol-C acetate was reduced by sodium borohydride to demethyl-hydroxy-alcohol-C acetate (VIII), presumably a mixture of two hydroxy epimers. Treatment of (VIII) with pyridine and phosphoryl chloride afforded a Beilstein test-positive product*[2] which was chromatographed and separated into a halogenated compound and a dehydrated one. The latter, though obtained in poor yield, showed bands at 845 and 821 cm^{-1} (-CH=C<) (in Nujol) in its infrared spectrum and was identical with cycloartenyl acetate (IX). Bayer-Villiger oxidation of demethyl-oxo-alcohol-C acetate with excess peracetic acid, followed by hydrolysis of the crude product, gave a hydroxy acid, characterized as its methyl ester and methyl ester acetate. The above hydroxy acid was identical with 3β-hydroxy-25,26,27-trisnorcycloartan-24-oic acid (X) derived from cycloartenyl acetate by standard methods. Although, isopropanol which would be obtainable as the fragmentary product was not isolated, the reaction can be explained by the presence of a carbonyl group at C-24 in demethyloxo-alcohol-C acetate.

It is concluded that alcohol-C is 24-methylenecycloartanol (III) or 9,19-cycloeburic-24(28)-en-3β-ol, and that oryzanol-C is 24-methylenecycloartanyl ferulate. Alcohol-C has the same side chain as cycloeucalenol[5~6] (4β-demethyl-24-methylenecycloartanol), and from the biogenetic view point they are closely related. Eburicoic acid and 24-methylenecholesterol also have a vinylidene group at C-24, but catalytic hydrogenation of the ethylenic linkage of the two compounds proceeds differently; eburicoic acid is hydrogenated to the 24b-methyl derivative,[7] whereas 24-methylenecholesterol is hydrogenated to campestanol (24a-methyl).[8] As shown in Table I of the preceding paper,[1] dihydroalcohol-C and its acetate differ from cyclolaudanol (24b-methyl) and its acetate, respectively. It has been shown[9] that introduction of a 24-methyl group into cholesterol and its derivatives causes an increment in molecular rotation; contribution of 24a-methyl is +22° and that of 24b-methyl is −29°. The configuration of C-24 in cyclolaudenol[3] and dihydroeburicoic acid[3,8] was determined by molecular rotational differences. When this method is applied to dihydroalcohol-C, as shown below, the molecular rotations of dihydroalcohol-C and its acetate are almost the same as those of cycloartanol and its acetate, respectively. Therefore, dihydroalcohol-C seems to be a mixture of 24a- and 24b-methyl epimers. Recently, similar results were reported in the reduction of cycloeucalenol.[6]

Table I.

	$[M]_D$*		$[M]_D$*
Cycloartanol	+195°	Dihydroalcohol-C	+205°
Cycloartanyl acetate	+268°	Dihydroalcohol-C acetate	+264°

* $[M]_D$ was calculated from the data obtained in the present experiments. Barton reported +193° for cycloartanol and +268° for its acetate (J. Chem. Soc., **1951**, 1444). Henry, *et al.*[3] reported +214° for cycloartanol and +277° for its acetate.

*[2] The same sequence has already been applied for the degradation of cycloeucalenyl acetate which contains C-24-methylene grouping and the corresponding dehydrated product has been reported to be analytically impure. cf. J. S. G. Cox, F. E. King, T. J. King : J. Chem. Soc., **1956**, 1384.

5) J. S. G. Cox, F. E. King, T. J. King : Proc. Chem. Soc., **1957**, 290.

6) *Idem.* : J. Chem. Soc., **1959**, 514.

7) J. M. Guider, T. G. Halsall, E. R. H. Jones : *Ibid.*, **1954**, 4471.

8) D. R. Idler, U. H. M. Fagerlund : J. Am. Chem. Soc., **77**, 4142(1955).

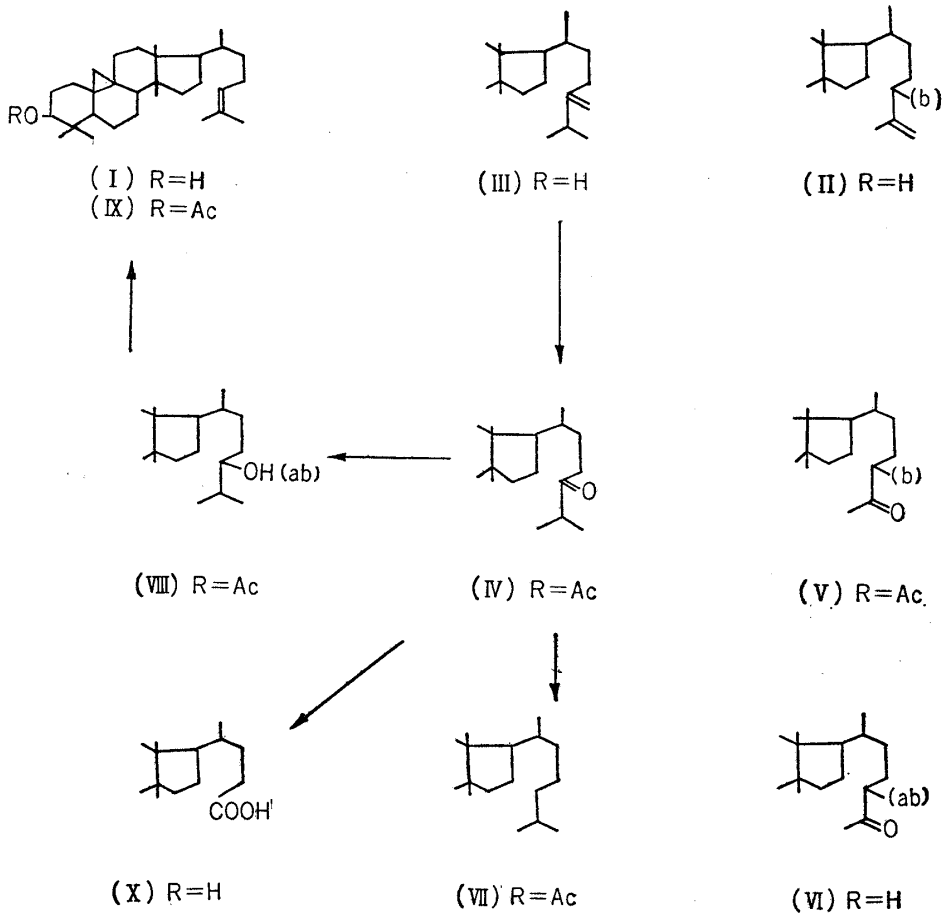
9) cf. L. F. Fieser, M. Fieser : "Natural Products related to Phenanthrene," 3rd Ed., 207(1949), Reinhold Publishing Corp.

〔Addendum〕 Dr. P. Crabbè kindly suggested that it is preferable to compare 24-methylenecycloartanol (alcohol-C) and cyclolaudenol directly, as their constants are similar. The direct comparison was kindly carried out by Prof. F. S. Spring, to whom a deep gratitude is expressed. The results given in Table II proved that the two compounds differ from each other.

Table II. Mixed m.p. Determination

	m.p.(°C)	Mixed m.p.(°C)		m.p.(°C)
1. Cyclolaudenol	124~125	117~119	24-Methylenecycloartanol	119~121
2. Cyclolaudenyl acetate	120~121	107~110 (cloudy to 114)	24-Methylenecycloartanyl acetate	114~115
3. Cyclolaudenyl benzoate	193~194	157~189	24-Methylenecycloartanyl benzoate	157~158
4. Cyclolaudenone	114~115	103~107	24-Methylenecycloartanone	108~109
5. 25-Oxo-26-norcyclo-24ab-laudanyl acetate	121.5~122.5	110~114	24-Oxocycloartanyl acetate	120~122

The infrared spectra of corresponding compounds listed above in 1, 2 and 5 were different.



Experimental

(All m.p.s are uncorrected. $[\alpha]_D$ taken in $CHCl_3$ unless otherwise stated)

Ozonolysis of Alcohol-C Acetate—Alcohol-C acetate (2.07 g.) in purified $CHCl_3$ (100 cc.) was treated at −50° with ozonised oxygen (2 moles of O_3) for 25 min. After removal of the cold bath, the solution was treated with AcOH (20 cc.) and Zn dust (12 g.), and stirred at room temperature until it no longer colored starch-KI solution. The filtered solution was washed with water (total 200 cc.). The aqueous washings were combined, adjusted to pH 6, and treated with 1% aqueous solution of dimedone (140

cc.). After 24 hr., the separated formaldehyde-dimedone compound (0.73 g., m.p. 185~189°) was collected and crystallized from hydr. EtOH, from which it separated as needles (0.61 g.), m.p. and mixed m.p. 189~190°. *Anal.* Calcd. for $C_{17}H_{24}O_4$: C, 69.83; H, 8.29. Found: C, 69.91; H, 8.06.

The filtrate from the dimedone derivative was steam distilled and the distillate was treated with aqueous solution of 2,4-dinitrophenylhydrazine hydrochloride but no hydrazone separated.

Evaporation of the dried $CHCl_3$ solution gave a solid which was dissolved in light petroleum (60 cc.) and chromatographed over alumina (50 g., deactivated with AcOH). Elution with light petroleum (180 cc.) and light petroleum-benzene (9:1, 60 cc.) gave a fraction (1.7 g.) which, after crystallization from $CHCl_3$-MeOH, gave demethyl-oxo-alcohol-C acetate (1.24 g.) as blades, m.p. 121~123°; $[\alpha]_D$ +52° (c=1.82). *Anal.* Calcd. for $C_{32}H_{52}O_3$: C, 79.28; H, 10.81. Found: C, 79.17; H, 10.63.

Oxime: Separated from ether-light petroleum as needles, m.p. 193~194°; $[\alpha]_D$ +50° (c=1.08). *Anal.* Calcd. for $C_{32}H_{53}O_3N$: C, 76.90; H, 10.69. Found: C, 76.90; H, 10.27.

Demethyl-oxo-alcohol-C-Demethyl-oxo-alcohol-C acetate (0.15 g.) was refluxed with 5% methanolic KOH solution (50 cc.) for 4 hr. to yield demethyl-oxo-alcohol-C, separating from MeOH as plates (0.09 g.), m.p. 92~94°,*3 $[\alpha]_D$ +44.5° (c=2.48). *Anal.* Calcd. for $C_{30}H_{50}O_2$: C, 81.38; H, 11.38. Found: C, 81.00; H, 11.09.

Acetylation of this alcohol regenerated demethyl-oxo-alcohol-C acetate, m.p. and mixed m.p. 121~123°, $[\alpha]_D$ +53° (c=1.32). The oxime of the acetate, m.p. and mixed m.p. 192~194°.

Wolff-Kishner Reduction of Demethyl-oxo-alcohol-C Acetate-A mixture of demethyl-oxo-alcohol-C acetate (0.26 g.), hydrazine hydrate (0.3 cc.), and monoethylene glycol (40 cc.) was heated, the solvent distilled off until the vapor temperature reached 190°, KOH (1.3 g.) was added, and the mixture was refluxed for 14 hr. The mixture was poured into water and the product, isolated by means of ether, was treated on a water bath with pyridine (2.0 cc.) and Ac_2O (5 cc.). The acetylated product was chromatographed over alumina (21 g.). The fraction (0.16 g.) eluted with light petroleum (100 cc.) was crystallized from $CHCl_3$-MeOH to give demethyl-alcohol-C acetate, identical with cycloartanyl acetate (see below), m.p. and mixed m.p. 132~133°, $[\alpha]_D$ +59.5° (c=1.20). The IR spectrum of demethyl-alcohol-C acetate was identical with that of cycloartanyl acetate. *Anal.* Calcd. for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56. Found: C, 81.82; H, 11.51.

Hydrolysis of demethyl-alcohol-C acetate (70 mg.) with 3% methanolic KOH solution (20 cc.) gave demethyl-alcohol-C separating from hydr. MeOH as blades, m.p. 99~101°, undepressed on admixture with cycloartanol (see below). The analytical sample, after drying *in vacuo*, melted at 100° (cloudy to 106°); $[\alpha]_D$ +46° (c=0.76). *Anal.* Calcd. for $C_{30}H_{50}O \cdot \frac{1}{2}CH_3OH$: C, 82.35; H, 12.25. Found: C, 82.27; H, 12.07.

Demethylhydroxy-alcohol-C Acetate—To a solution of demethyl-oxo-alcohol-C acetate (0.72 g.) in EtOH (80 cc.), a solution of $NaBH_4$ (0.20 g.) in MeOH (10 cc.) was added and the mixture was kept at room temperature for 3 hr. After destroying excess $NaBH_4$ with AcOH, the mixture was concentrated *in vacuo* and water was added. The product, isolated by means of ether, was dissolved in light petroleum and chromatographed over alumina (21 g.). Fractions eluted with light petroleum-benzene (9:1, 40 cc., and 1:1, 40 cc.) was crystallized from MeOH to yield demethylhydroxy-alcohol-C acetate as needles (0.57 g.), m.p. 128~132°, $[\alpha]_D$ +27° (c=1.97). *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 78.64; H, 10.87.

Dehydration of Demethylhydroxy-alcohol-C Acetate—Demethylhydroxy-alcohol-C acetate (0.24 g.) was dissolved in pyridine (2 cc.) and treated with a solution of $POCl_3$ (0.8 cc.) in the same solvent (2 cc.) at 100° for 1 hr. The mixture was poured into ice water (30 cc.) and extracted with ether. The evaporated residue of neutral ethereal solution gave positive Beilstein test and was chromatographed through alumina (12 g.). The chromatogram was eluted with (a) light petroleum (30 cc.), (b) light petroleum (50 cc.), (c) light petroleum (40 cc.) and light petroleum-benzene (9:1, 30 cc.), and (d) light petroleum-benzene (9:1, 20 cc.). Compounds from fraction (c), m.p. 110~114° (75 mg.), and from (d), m.p. 130~136° (50 mg.), giving positive Beilstein test, were not examined. A compound of m.p. 118~121° (70 mg.) from fraction (b) was again chromatographed and the product was crystallized from $CHCl_3$-MeOH to give a dehydrated compound as plates (25 mg.), m.p. 121.5~123°; $[\alpha]_D$ +55°, (c=0.94). This was identical with cycloartenyl acetate and undepressed on admixture. The IR curves of the two compounds were identical (in Nujol). *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.97; H, 10.86.

Bayer-Villiger Oxidation of Demethyl-oxo-alcohol-C Acetate-A mixture of demethyl-oxo-alcohol-C acetate (0.45 g.), *p*-toluenesulfonic acid (10 mg.), and 5% peracetic acid (15 cc.) was heated at 40~50° for 8 hr. After dilution with ether, the mixture was washed with water, dilute Na_2CO_3 solution, and water. The ether layer was dried and evaporated. The residue was refluxed with 5% methanolic KOH solution (20 cc.) for 2 hr., the solvent was concentrated *in vacuo*, and shaken with water

*3 Oxo-norcyclo-24ab-laudanol,[3] m.p. 139~141°, and its acetate oxime, m.p. 153~154°, are clearly different from demethyl-oxo-alcohol-C and its acetate oxime, respectively.

and ether. A salt separating at the solvent interface was collected, suspended in water, acidified with hydrochloric acid, and the resulting acid compound was collected by means of ether. One crystallization of the crude acid (0.36 g.) from acetone gave plates of m.p. 215~220° (0.19 g.). Two more crystallizations from the same solvent gave analytically pure sample, m.p. 220~222°; $[\alpha]_D$ +49° (c=0.74, in pyridine). *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.91; H, 10.61. The acid did not depress the m.p. of 3β-hydroxy-25,26,27-trisnorcycloartan-24-oic acid (see below) and the IR curves of the two specimens were identical.

With ethereal CH_2N_2, the acid gave a methyl ester of blades (from $CHCl_3$-MeOH), m.p. 131~132°, $[\alpha]_D$ +42° (c=0.78). *Anal.* Calcd. for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 78.55; H, 10.79.

Acetylation of the methyl ester gave methyl ester acetate as needles (from $CHCl_3$-MeOH); $[\alpha]_D$ +54° (c=1.18); m.p. 121.5~122.5°, alone or mixed with methyl 3β-acetoxy-25,26,27-trisnorcycloartan-24-oate (see below). *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.19; H, 10.27.

Cycloartanol—Cycloartenyl acetate (0.65 g.) in AcOH (100 cc.) was hydrogenated over Pt catalyst. Usual treatment and crystallization from $CHCl_3$-MeOH afforded cycloartanyl acetate (0.58 g.) as needles, m.p. 132~133°; $[\alpha]_D$ +57° (c=1.27), $[M]_D$ +268°. *Anal.* Calcd. for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56. Found: C, 81,24; H, 11.25. (reported m.p. 132~133°, $[\alpha]_D$ +57°[10]; m.p. 130~132°, $[\alpha]_D$ +59°[11]).

Hydrolysis of the acetate with 3% methanolic KOH solution gave cycloartanol as plates (from MeOH), m.p. 99~101°, which, after drying *in vacuo*, melted at 100~107°; $[\alpha]_D$ +45.5° (c=1.42), $[M]_D$ +195°. *Anal.* Calcd. for $C_{30}H_{52}O \cdot \frac{1}{2}CH_3OH$: C, 82.35; H, 12.25. Found: C, 82.32, 82.61; H, 12.51, 12.12. (reported m.p. 99~101°, $[\alpha]_D$ +45°[10]; m.p. 99°, $[\alpha]_D$ +50°[11]). IR cm^{-1}: 3600, 3040, 1045, 1022, 1004, 988.

3β-Hydroxy-25,26,27-trisnorcycloartan-24-oic Acid—Cycloartenyl acetate (0.50 g.) in purified $CHCl_3$ (30 cc.) was treated at −50° with ozonised oxygen (2 moles of O_3). After treating with Zn dust (2 g.) and AcOH (5 cc.), the solution was filtered, washed with water, dried, and evaporated. The residue was dissolved in AcOH (10 cc.), added with a solution of CrO_3 (0.20 g.) in water (2 cc.), and the mixture was allowed to stand at room temperature over night. After dilution with water the mixture was shaken with ether. The ethereal solution was washed with water and 5% K_2CO_3 solution, the solid that separated at the interface was collected, shaken with HCl, and extracted with ether. Evaporation of the ethereal solution and crystallization of the residue (0.35 g.) from acetone afforded 3β-acetoxy-25,26,27-trisnorcycloartan-24-oic acid as plates (0.20 g.), m.p. 217~219°; $[\alpha]_D$ +59° (c=1.20). *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.74; H, 10.06 (reported m.p. 221.5~223°, $[\alpha]_D$ +62°[11]).

With ethereal CH_2N_2 this gave methyl ester acetate, needles (from $CHCl_3$-MeOH), m.p. 121~122°; $[\alpha]_D$ +54° (c=1.28) (reported m.p. 121~123°, $[\alpha]_D$ +56°[11]). *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.32; H, 10.32.

Hydrolysis of the above acetoxy acid (0.1 g.) with 5% methanolic KOH solution (10 cc.) gave 3β-hydroxy-25,26,27-trisnorcycloartan-24-oic acid as plates (from acetone), m.p. 220~222°; $[\alpha]_D$ +48.5° (c=0.80, in pyridine). *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.98; H, 10.71.

The writer expresses his deep gratitude to Dr. Junzo Shinoda, President of this Company, to Mr. Sakan Hashimoto, Director of this Laboratory, for kind encouragement, and to Dr. Masao Shimizu, Acting Director, for kind guidance. The writer is also indebted to Messrs. Kurihara and Abe for the elemental analyses.

Summary

Alcohol-C, obtained by saponification of oryzanol-C, is related to cycloartenol. Ozonolysis of alcohol-C acetate gave formaldehyde and demethyl-oxo-alcohol-C acetate (IV). Wolff-Kishner reduction of (IV) yielded cycloartanol while reduction of carbonyl group of (IV) to the corresponding hydroxyl compound, followed by dehydration, gave cycloartenyl acetate. (IV) was converted to trisnorcycloartanolic acid (X) by Bayer-Villiger oxidation. From the result of these reactions, alcohol-C would be represented as 24-methylenecycloartanol (III).

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10) D. H. R. Barton: J. Chem. Soc., **1951**, 1444.
11) H. R. Bentley, J. A. Henry, D. S. Irvine, F. S. Spring: *Ibid.*, **1953**, 3673.