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# Chemical Studies on the Oriental Plant Drugs. XXVI.[1] Saponins and Sapogenins of *Ginseng*. The Absolute Configurations of Cinenic Acid and Panaxadiol[2]

MASAHIRO NAGAI,[3a] OSAMU TANAKA,[3b] and SHOJI SHIBATA[3c]

*Faculty of Pharmaceutical Sciences, University of Tokyo*[3]

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(−) Cinenic acid was obtained by stepwise degradation of panaxadiol (I). The R-configuration of (−) cinenic acid (II) and its methyl ester (X) was established by the derivation from R-(−) linalool. Consequently, the absolute configuration at $C_{(20)}$ of panaxadiol was proved to be R.

Panaxadiol (I) has been proved to be an artifact which is formed from the genuine sapogenin having an open side chain during the process of hydrolysis with ethanolic mineral acid of ginsenosides-$Rb_1$, $Rb_2$ and Rc, the neutral saponins of *Ginseng* root.[4,5] The stereochemistry at C-20[6] of I has not fully been established.

This paper deals with the elucidation of the absolute configuration at C-20 of panaxadiol (I), which in connection with our subsequent paper,[7] leads to the absolute configuration at C-20 of dammarane type triterpenes.

Optically active cinenic acid (II) has been obtained from I by the following degradation reactions: The hydroxyl group at C-3 of I was selectively oxidized with chromic acid in pyridine to yield the 3-keto derivative (III), (mp 236.5—238.5°), IR $\nu_{\max}^{\mathrm{CS_2}}$ 1715 (C=O) and 3396 $\mathrm{cm^{-1}}$ (12$\beta$-OH, intramolecularly hydrogen bonded with the oxygen of the trimethyltetrahydropyran ring), which on the Huang–Minlon reduction, yielded 3-desoxypanaxadiol (IV), (mp 192—193.5°), IR $\nu_{\max}^{\mathrm{CCl_4}}$ 3392 $\mathrm{cm^{-1}}$ (12$\beta$-OH) and no carbonyl band. Oxidation of IV with Jones' reagent afforded the 12-keto derivative (V), (mp 191—192°), IR $\nu_{\max}^{\mathrm{CCl_4}}$ 1709 $\mathrm{cm^{-1}}$ (C=O) and no hydroxyl band, which was subjected to the Baeyer–Villiger oxidation with permaleic acid or trifluoroperacetic acid to give a lactone, (mp 195—197°), IR $\nu_{\max}^{\mathrm{KBr}}$ 1735 $\mathrm{cm^{-1}}$ (lactone). The nuclear magnetic resonance (NMR) spectrum[8] of this lactone (4.82 (1H, doublet $J$=6.0 Hz, -CO-O-$\overset{|}{\mathrm{C}}\underline{\mathrm{H}}$-$\overset{|}{\mathrm{C}}$H-)) was consistent with the structure VI. Alkaline hydrolysis of VI followed by methylation of the resulted acid with diazomethane yielded an amorphous methyl ester (VII), IR $\nu_{\max}^{\mathrm{CCl_4}}$ 1745, 1163 (ester), and 3480 $\mathrm{cm^{-1}}$ (13-OH, intramolecularly hydrogen bonded). Several attempts to oxidize the hydroxyl group at C-13 to ketone were unsuccessful owing to the ready regeneration of the lactone (VI) from VII. Dehydration of the methyl ester (VII) with thionyl chloride afforded an oily product (VIII), IR $\nu_{\max}^{\mathrm{CCl_4}}$ 1746, 1167 $\mathrm{cm^{-1}}$ (ester) and no hydroxyl band, NMR 5.49 (1H singlet, -$\overset{|}{\mathrm{C}}\underline{\mathrm{H}}$=C-) and 3.70 (3H singlet,

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[1] Part XXV: T. Ando, O. Tanaka, and S. Shibata, *Japan J. Pharmacog.* **25**, 28 (1971).

[2] Preliminary communication of this study, *Tetrahedron Letters*, **1966**, 4797.

[3] Location: *a) Ebara 2-4-41, Shinagawa-ku, Tokyo,* Present address: *Hoshi College of Pharmacy*; *b) Kasumi 1-2-3, Hiroshima,* Present address: *Pharmaceutical Institute, School of Medicine, Hiroshima University*; *c) Hongo, Bunkyo-ku, Tokyo.*

[4] M. Fugita, S. Itokawa, and S. Shibata, *Yakugaku Zasshi*, **82**, 1634, 1638 (1962).

[5] S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima, and T. Ohsawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 595 (1966); Preliminary report, *Tetrahedron Letters*, **1963**. 795.

[6] O. Tanaka, M. Nagai, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **14**, 1150 (1966), Preliminary report, *Tetrahedron Letters*, **1964**, 2291, and the references cited therein.

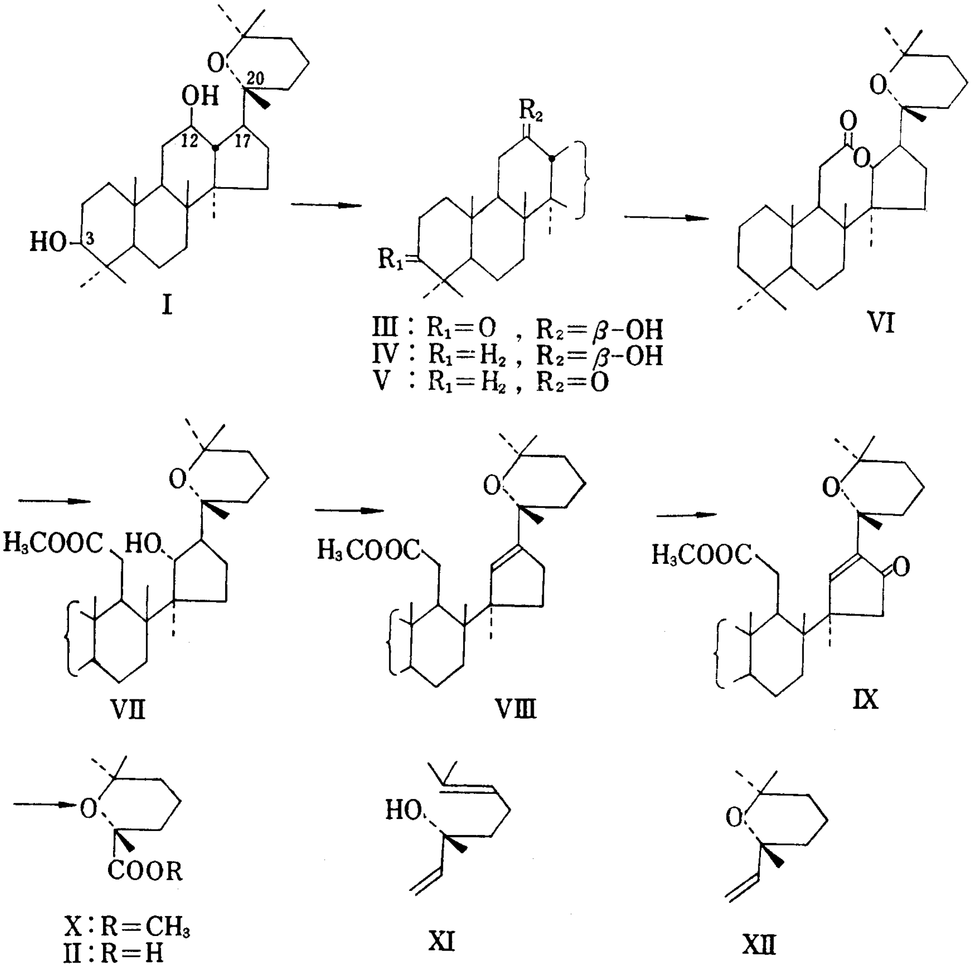
[7] M. Nagai, O. Tanaka, T. Ohsawa, N. Tanaka, K. Kawai, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), to be submitted; Preliminary report, *Tetrahedron Letters*, **1967**, 391.

[8] NMR spectra were determined in $CDCl_3$ solution and chemical shifts ($\delta$) are expressed in ppm from TMS.

-COOC$\underline{\underline{H}}_3$), which on subsequent oxidation with *tert*-butyl chromate gave an amorphous unsaturated keto ester (IX), IR $\nu_{max}^{CCl_4}$ 1742, 1167 (ester), 1698 and 1625 cm$^{-1}$ (-CH=$\overset{|}{C}$-C=O), UV $\lambda_{max}^{EtOH}$ 235 m$\mu$ ($\varepsilon$ 10.900), NMR 7.45 (1H singlet, -CH=$\overset{|}{C}$-CO-). This $\alpha,\beta$-unsaturated keto ester (IX) was oxidized with potassium permanganate in pyridine and the acidic fraction of the reaction product was extracted with *n*-hexane. The *n*-hexane-soluble part was methylated with diazomethane and the resulted crude methyl ester was purified by the preparative gas chromatography to give oily (−)-methyl cinenate (X), $[\alpha]_{590}^{14}$ −12.3°, $[\alpha]_{400}^{14}$ −49°, $[\alpha]_{340}^{14}$ −103° ($c$=0.2, CHCl$_3$).9) The structure of X was confirmed by the comparison of the infrared (IR) spectrum, and the retention time of the gas chromatogram with those of racemic methyl cinenate prepared from 1,8-cineol.10)

The absolute configuration of (−) cinenic acid (II) and its methyl ester (X) was elucidated by the derivation from (−) linalool (XII), whose absolute configuration has already been established to be R by Cornforth *et al.*11)

R-(+)-2,2,6-Trimethyl-6-vinyltetrahydropyran (XII), which has been revealed to retain the same absolute configuration as that of R (−) linalool (XI), was prepared from (XI) by the procedure reported in the literature.12) Oxidation of XII with KMnO$_4$ and



I

III : R$_1$=O , R$_2$=$\beta$-OH
IV : R$_1$=H$_2$ , R$_2$=$\beta$-OH
V : R$_1$=H$_2$ , R$_2$=O

VI

VII

VIII

IX

X : R=CH$_3$
II : R=H

XI

XII

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9) Optical activities (ORD curves) were measured with Spectrophotometer Model ORD/UV-5, Japan Spectroscopic Co., Ltd.

10) H. Rupe, and A. Blechschmidt, *J. Prakt. Chem.*, (ii) **96**, 59 (1917).

11) R.H. Cornforth, J.W. Cornforth, and V. Prelog, *Ann. Chem.* **634**, 197 (1960); G. Ohloff, and E. Klein, *Tetrahedron* **18**, 37 (1962).

12) E. Klein, H. Farnow, and W. Rojahn, *Ann. Chem.*, **675**, 73 (1964); D. Felix, A. Melera, J. Seible, and E. sz. Kovàts, *Helv. Chem. Acta.*, **46**, 1513 (1963); H. Stickler, G. Ohloff, and E. sz. Kovàts, *Tetrahedron Letters*, **1964** 649.

$NaIO_4$[13] afforded (—) cinenic acid (II), $[\alpha]^{14}_{590}$ $-7.3°$, $[\alpha]^{14}_{400}$ $-26.0°$, $[\alpha]^{14}_{340}$ $-54.8°$ ($CHCl_3$),[9] whose IR spectrum and *Rf* value of the thin-layer chromatogram were identical with those of racemic cinenic acid.[10] (—) Cinenic acid was methylated with $CH_2N_2$ to give oily (—) methyl cinenate (X), $[\alpha]^{29}_{590}$ $-10.1°$, $[\alpha]^{29}_{400}$ $-37.6°$, $[\alpha]^{29}_{340}$ $-81.8°$ ($CHCl_3$),[9] whose IR spectrum and the retention time of the gas chromatogram were idetical with those of racemic methyl cinenate.[10] The absolute configuration of (—) cinenic acid (II) as well as (—)-methyl cinenate (X) can be assigned as R. Consequently the configuration at C-20 of panaxadiol (I) should also be represented by R.[14] The lower optical activity of methyl cinenate prepared from XI than that obtained from I would be due to the partial racemization during the process of the derivation.

## Experimental[15]

**Oxidation of Panaxadiol (I) with Chromic Acid in Pyridine**——To a solution of I (0.2 g) in pyridine (6 ml) was added a solution of $CrO_3$ (0.24 g) in pyridine (4 ml) and the reaction mixture was allowed to stand at room temperature for 1 day. After working up in the usual way, the product was recrystallized from acetone to give the 3-keto derivative (III), colorless crystals (0.13 g), mp 236.5—238.5°, $[\alpha]^{24.5}_{D}$ $+24.7°$ ($c=1.24$, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.72; H, 10.99.

**The Huang-Minlon Reduction of III**——A solution of III (12 g) in ethanol (70 ml) was added to a solution of 80% hydrazine hydrate (6.5 ml) and KOH (4.0 g) in diethyleneglycol (160 ml). The mixture was heated under reflux for 1.5 hr and then distilled until the temperature reached 220°, and then the mixture was kept at 230—240° for additional 4 hr. To the cooled reaction mixture was added aqueous solution of NaCl (500 ml), and the product was isolated with benzene. The crude product (11.6 g) was recrystallized from methanol yielding 3-desoxypanaxadiol (IV), colorless crystals, mp 192—193.5°, $[\alpha]^{20}_{D}$ $+2.2°$ ($c=1.3$, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 81.25; H, 11.86.

**Oxidation of IV with Jones' Reagent**——To a solution of IV (0.95 g) in acetone (100 ml) was added dropwise Jones' reagent until the reddish orange color of the reagent continued for 2 hr, keeping the temperature below 25°. The crude product was recrystallized from methanol to give the 12-keto derivative (V) (0.82 g), mp 191—192°, $[\alpha]^{20}_{D}$ $+5.5°$ ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.35; H, 11.33.

**The Baeyer-Villiger Oxidation of V**——*a*) With Permaleic Acid:[16] A solution of V (10 g) in anhyd. $CH_2Cl_2$ (65 ml) was added dropwise under ice cooling to a solution of permaleic acid in anhyd. $CH_2Cl_2$ (65 ml) prepared from maleic anhydride (12.3 g) and 90% $H_2O_2$ (10 g). The reaction mixture was then refluxed for 2 hr. After cooling, the resulted precipitate (maleic acid) was removed by filtration and washed with $CH_2Cl_2$. The filtrate and the washing were combined and washed successively with water, 2N NaOH aqueous solution, $NaHSO_3$ aqueous solution, and water. Concentration of this solution to dryness and recrystallization of the residue from methanol afforded the lactone (VI) (9.1 g), mp 196—198°, $[\alpha]^{23}_{D}$ $+57.6°$ ($c=1.66$, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.62; H, 10.76.

*b*) With Trifluoroperacetic Acid: To a mixture of V (0.8 g) and anhyd. $Na_2HPO_4$ (4.9 g) in anhyd. $CH_2Cl_2$ (12 ml) was added dropwise under ice cooling a solution of trifluoroperacetic acid in $CH_2Cl_2$ (7 ml) prepared from trifluoroacetic anhydride (2.31 g) and 90% $H_2O_2$ (0.38 ml). The reaction mixture was then allowed to stand at room temperature for 1 hr. After working up in the usual way, the product was recrystallized from methanol to yield VI (0.73 g), mp 196—198°.

**The $\alpha,\beta$-Unsaturated Keto Ester (IX) from VI through the Methyl Ester (VII) and VIII**——The lactone (5.0 g) was refluxed in aqueous methanolic KOH (KOH 37.5 g, methanol 450 ml, and water 50 ml) for 6 hr. The solution was concentrated to about 60 ml *in vacuo*, poured into ice water, and neutralized carefully[17] with 10% aqueous acetic acid. The precipitate was taken up in ether, and the ether layer was washed with water and dried over anhyd. $Na_2SO_4$. To this ethereal solution was added a solution of diazomethane in ether and the reaction mixture was kept at room temperature for 5 hr. Concentration to dryness gave

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[13] R.U. Lemieux, and E. von Rudloff, *Canad. J. Chem.*, **33**, 1710 (1966).

[14] H. Stickler, and G. Ohloff also established the R configuration of XI at the almost same time with our preliminary report[2] (*Helv. Chem. Acta.*, **49**, 2157 (1966)).

[15] All melting points are uncorrected and were determined on a Kofler block.

[16] W.D. Emmons, G.B. Lucas, *J. Am. Chem. Soc.*, **77**, 2287 (1955); R.W. White, W.D. Emmons, *Tetrahedron*, **17**, 31 (1962).

[17] If the acidity exceeds pH=6.5, the remarkable reproduction of the lactone (VI) was observed. The methyl ester (VII) also reproduced the lactone (VI) on standing its methanolic solution for a long time at room temperature.

amorphous methyl ester (VII). Oxidations of VII with *tert*-butyl chromate, Jones' reagent, or chromic acid in pyridine afforded no ketonic compound but regenerated the lactone (VI).

To a solution of the methyl ester (VII) (0.67 g) in pyridine (6 ml) was added $SOCl_2$ (0.5 ml) under stirring and cooling with a mixture of ice and NaCl. The reaction mixture was held at room temperature for 1.5 hr and finally heated at 80° for 5 min. After working up in the usual way, the crude product was subjected to chromatography on silica gel: gradient elution with *n*-hexane–benzene–ether. From the eluates with mixtures of *n*-hexane–benzene (1: 1 and 2: 3), an oily substance was obtained (VIII) (0.3 g), NMR in $CDCl_3$: 5.49 (1H singlet), 3.70 (3H singlet), 1.20 (9H singlet), 0.94 (3H singlet), 0.89 (9H singlet), and 0.82 (3H singlet), which was shown to be homogeneous by thin layer chromatography on silica gel using a mixture of benzene and $CHCl_3$ (2: 1) as a developing solvent. From the eluate with a mixture of benzene and ether (9: 1), the lactone (VI) was obtained.

To a solution of VIII (1.3 g) in anhyd. $CCl_4$ (10 ml) was added a solution of *tert*-butyl chromate[18] (0.22 g $CrO_3$/ml) in a mixture of acetic acid and acetic anhydride (15 ml) at 50° for a period of 1 hr, and the reaction mixture was kept at the same temperature for 6 hr. After decomposition of the excess reagent by the addition of an aqueous solution of $NaHSO_3$, the reaction mixture was extracted with ether. The ether layer was washed successively with water, 2N NaOH, and water, dried over anhyd. $Na_2SO_4$ and evaporated to dryness and the resulted oily residue was subjected to chromatography on silica gel to give the $\alpha,\beta$-unsaturated keto ester (IX) (0.5 g), NMR in $CDCl_3$: 7.45 (1H singlet), 3.69 (3H singlet), a pair of AB type doublets centered at 2.35 ($J=19.8$ Hz), 1.33 (3H singlet), 1.23 (3H singlet), 1.17 (3H singlet) 1.10 (3H singlet), 0.91 (3H singlet), 0.85 (6H singlet), and 0.78 (3H singlet).

**(−) Methyl Cinenate from IX**——A solution of $KMnO_4$ (1.0 g) in water (5.5 ml), pyridine (5 ml), and 10% aqueous NaOH (2 ml) was gradually added to a solution of IX (0.35 g) in pyridine (9 ml), and the reaction mixture was heated at 80° for 1 hr under occasional stirring. After decomposition of $MnO_2$ and excess of $KMnO_4$ by careful addition of aqueous $NaHSO_3$ solution to the acidified reaction mixture, aqueous NaCl solution was added to the reaction mixture. The product was taken up in ether, and the ether layer was washed with aqueous 2N HCl solution and water, and then extracted with aqueous 2N $Na_2CO_3$ solution. The resulted $Na_2CO_3$ layer was acidified and extracted with ether. After washing with water and drying over anhyd. $Na_2SO_4$, the ethereal solution was concentrated to dryness to give an oily residue, which was extracted with *n*-hexane. The presence of cinenic acid in the *n*-hexane soluble part was shown by thin-layer chromatography on silica gel impregnated with oxalic acid (solvent: $CHCl_3$). The crude acid was methylated with diazomethane in the usual manner, and the resulted methyl ester was separated by preparative gas liquid chromatography[19] to give oily (−)-methyl cinenate (X), ORD ($c=0.2$, $CHCl_3$) $[\alpha]^{14}_{590}$ $-12.3°$, $[\alpha]^{14}_{400}$ $-49°$, $[\alpha]^{14}_{340}$ $-103°$, $[\alpha]^{14}_{300}$ $-188°$. The identity of X with the authentic sample of racemic methyl cinenate was established by the comparison of IR spectra and the retention time of gas chromatograms (Condition: Column, 12% Diethyleneglycol succinate on chromosorb W (4 mm). Column temperature, 103°, sample heater temperature, 180°, carrier gas, $N_2$ (45 ml/min.). on Shimadzu gaschromatograph GC-1B with hydrogen flame ionization detector).

**Oxidation of R (+)-2,2,6-Trimethyl-6-vinyltetrahydropyran (XII)**——Oxidation of racemic 2,2,6-trimethyl-6-vinyltetrahydropyran to racemic cinenic acid with $KMnO_4$ in alkaline medium has been reported by Ohta, *et al.*[20] The present authrors found that better yield of (−)-cinenic acid from XII (1.0 g) was obtained by the Lemieux oxidation.[13] To a mixture of XII prepared from R-(−)-linalool (XI),[12] $Na_2CO_3$ (0.3 g), pyridine (3 ml), and water (3.0 ml) was added a solution of $KMnO_4$ (0.05 g) and $NaIO_4$ (6.5 g) in water (50 ml) under stirring and cooling with ice water, and the mixture was held at room temperature under stirring overnight. The reaction mixture was acidified under ice cooling and the resulting precipitate was taken up in ether. After washing with aqueous $NaHSO_3$ solution, the ether layer was extracted with aqueous 2N $Na_2CO_3$ solution and the alkaline layer was concentrated *in vacuo* and acidified under cooling. The acidified solution was extracted with ether and the ether layer was washed with water, dried, and concentrated to dryness to give a crystalline residue (0.98 g), mp 41—46°, which was recrystallized from water affording R-(−)-cinenic acid (II), mp 47.5—48.5°, ORD: ($c=6.57$, $CHCl_3$) $[\alpha]^{27}_{590}$ $-7.3°$, $[\alpha]^{27}_{400}$ $-26.0°$, $[\alpha]^{27}_{340}$ $-54.8°$, $[\alpha]^{27}_{300}$ $-106°$. The IR spectrum of II in $CCl_4$ and the *Rf* value of II of the thin-layer chromatogram on silica gel impregnated with oxalic acid (solvent $CH_2Cl_2$) were completely identical with those of authentic sample of racemic cinenic acid.

**R-(−)-Methyl Cinenate (X) from II**——R-(−)-Cinenic acid (XI) (0.4 g) was treated with diazomethane in ether. The product was purified by distillation (14 mmHg, external temperature 110°) to give X, colorless oil (0.36 g), ORD ($c=2.13$, $CHCl_3$) $[\alpha]^{29}_{590}$ $-10.1°$, $[\alpha]^{29}_{400}$ $-37.6°$, $[\alpha]^{29}_{340}$ $-81.8°$, $[\alpha]^{29}_{300}$ $-158°$. The identification of X was achieved by the comparison of IR spectrum and the retention time of the gas chromatogram (Condition; loc. cit.) with those of authentic racemic methyl cinenate.

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18) K. Heusler, and A. Wettstein, *Helv. Chem. Acta*, 35, 289 (1952).

19) Condition: Column, 20% diethylene glycol succinate on Chromosorb W (4 m), column temperature 130°, sample heater temperature 198°, Carrier gas $N_2$ on Shimadzu gas chromatograph Model GC-1C.

20) Y. Ohta, K. Nishimura, and Y. Hirose, *Agr. Biol. Chem.*, 28, 5 (1964).

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