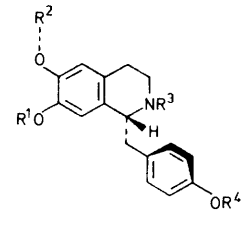
Biosynthesis of Coclaurine

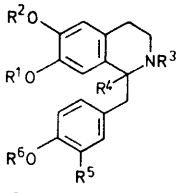
By **Om Prakash, Dewan S. Bhakuni,** and **Randhir S. Kapil,*** Central Drug Research Institute, Lucknow 226001, India

Feeding of tyrosine, tyramine, dopa, dopamine, and 4-hydroxyphenylpyruvic acid has been examined in the biosynthesis of coclaurine in *Annona reticulata*. While tyramine, dopa, and dopamine contribute to the formation of the phenethylamine portion of coclaurine, tyrosine and 4-hydroxyphenylpyruvic acid are incorporated into both halves. Tracer experiments show that coclaurine is biosynthesised *via* the intermediacy of norcoclaurine-1-carboxylic acid, 1,2-didehydronorcoclaurine, and norcoclaurine.

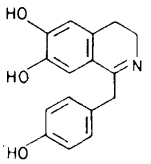
COCLAURINE, an established precursor of proaporphine,[1,2] aporphine,[3] and bisbenzylisoquinoline [4-6] alkaloids, has been assigned structure (1) (undefined stereochemistry) which has been confirmed by several syntheses.[7-9] *X*-Ray analysis of coclaurine hydrochloride monohydrate has revealed that the (+)-enantiomer has the D-configuration [10] (1), while Barton *et al.*[1] have reported the L-configuration for (−)-coclaurine.



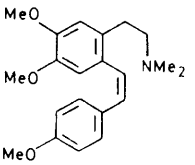
(1) $R^1 = R^3 = R^4 = H, R^2 = Me$
(2) $R^1 = R^2 = R^3 = R^4 = H$
(3) $R^1 = R^2 = R^3 = R^4 = Me$



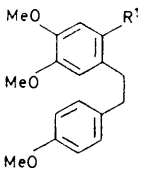
(4) $R^1 = R^2 = R^3 = R^5 = R^6 = H, R^4 = CO_2H$
(5) $R^1 = R^4 = H, R^2 = R^3 = R^6 = Me, R^5 = OH$



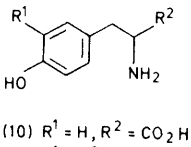
(6)



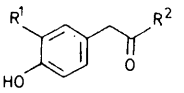
(7)



(8) $R^1 = [CH_2]_2 NMe_2$
(9) $R^1 = CH:CH_2$



(10) $R^1 = H, R^2 = CO_2H$
(11) $R^1 = R^2 = H$
(12) $R^1 = OH, R^2 = CO_2H$
(13) $R^1 = OH, R^2 = H$



(14) $R^1 = R^2 = H$
(15) $R^1 = H, R^2 = CO_2H$

The biosynthesis of coclaurine could be envisaged from norcoclaurine (2) which in turn could originate by interaction of dopamine (13) with 4-hydroxyphenylacetaldehyde (14) or 4-hydroxyphenylpyruvic acid (15), each derivable from tyrosine.[11] If (15) is the precursor, norcoclaurine-1-carboxylic acid (4) should be an obligatory intermediate in the biosynthesis of (1).

Our recent work on the biosynthesis of the 1-benzyltetrahydroisoquinoline system, as exemplified by reticuline (5),[12] has shown that the latter is formed in *Litsea glutinosa* (Lour.) C.B.Rob. (Lauraceae) by condensation of dopamine and 3,4-dihydroxyphenylpyruvic acid. Norlaudanosoline-1-carboxylic acid was found to be the precursor of reticuline, norlaudanosoline,[13] and morphine.[14] The *Papaver* species also converted 1,2-didehydronorlaudanosoline into morphine alkaloids.[14] Further, tyrosine has been demonstrated to give rise to two different constituent units of 1-benzyltetrahydroisoquinoline-derived alkaloids by independent pathways,[15] whereas dopa (12) and dopamine have been shown to be the specific precursors of only the phenethylamine portion of reticuline,[12] glaucine,[16] and morphine.[17]

We have now shown for the biosynthesis of coclaurine in *Annona reticulata* Linn. (Annonaceae) that dopamine and 4-hydroxyphenylpyruvic acid (derived from dopa and tyrosine, respectively) interact and form norcoclaurine-1-carboxylic acid which specifically gives rise to coclaurine *via* 1,2-didehydronorcoclaurine (6) and norcoclaurine.

(−)-[U-^{14}C]Tyrosine (experiment 1) (Table) was fed to young shoots of *A. reticulata* and it was found that the plants were actively biosynthesising coclaurine. Subsequently, feeding with (±)-[2-^{14}C]tyrosine (experiment 2) and (±)-[2-^{14}C]dopa (experiment 5) demonstrated that both these compounds are efficient precursors of (1). The position of the label in coclaurine derived from feeding [2-^{14}C]tyrosine was established as follows. (1) was converted into *NOO*-trimethylcoclaurine (3) by treatment with diazomethane followed by *N*-methylation with

Tracer experiments on *A. reticulata*

Expt.	Precursor fed	% Incorporation into coclaurine (1)
1	(−)-[U-^{14}C]Tyrosine (10)	0.70
2	(±)-[2-^{14}C]Tyrosine (10)	0.65
3	[3,5-$^{3}H_2$]Tyramine (11)	0.46
4	[3′,5′,3,3-$^{3}H_4$]-4-Hydroxyphenylpyruvic acid (15)	0.025
5	(±)-[2-^{14}C]Dopa (12)	0.42
6	[1-^{14}C]Dopamine (13)	1.2
7	(±)-[3-^{14}C]Norcoclaurine-1-carboxylic acid (4)	0.088
8	[1-^{14}C]1,2-Didehydronorcoclaurine (6)	0.19
9	(±)-[1-^{3}H]Norcoclaurine (2)	0.25

formaldehyde and formic acid. Conversion of (3) to its methiodide was achieved by refluxing with methyl iodide in methanol. Hofmann elimination of the methiodide gave the olefin (7) which on catalytic hydrogenation yielded the phenethylamine derivative (8) with no loss of activity. A second Hofmann degradation furnished the olefin (9) which on ozonolysis gave formaldehyde, trapped as its dimedone derivative (48.9% of original activity). The labelled coclaurine derived from feeding with [2-^{14}C]dopa was similarly degraded to afford formaldehyde dimedone (98% of original activity).

In a parallel experiment [3,5-$^{3}H_2$]tyramine (experiment 3) was found to be incorporated into (1). The labelled coclaurine was converted into *NOO*-trimethylcoclaurine. Hofmann elimination gave the olefin (7) with no loss of activity. Controlled potassium permanganate oxidation of (7) furnished *p*-anisic acid (radioinactive). These results indicated that the benzylic half of coclaurine was not derived from tyramine. [3′,5′,3,3-$^{3}H_4$]-4-Hydroxyphenylpyruvic acid (experiment 4), when fed to *A. reticulata* plants, was also found to be incorporated into (1). The labelled coclaurine was similarly degraded to *p*-anisic acid (25.1% of original activity). These results thus show that (15) is incorporated into both halves of (1). Feeding of [1-^{14}C]dopamine (experiment 6) demonstrates that (13) contributes to the phenethyl portion of coclaurine.

Feeding of (±)-[3-^{14}C]norcoclaurine-1-carboxylic acid (experiment 7) establishes the intermediacy of (4) in the biosynthesis of (1). The labelled coclaurine was degraded to methine (9) with no loss of activity. Ozonolysis of (9) gave formaldehyde dimedone (98.3% of original activity). Finally, feeding of [1-^{14}C]-1,2-didehydronorcoclaurine (6) (experiment 8) and [1-^{3}H]norcoclaurine (experiment 9) to *A. reticulata* plants showed that probably (6) is reduced to (2) before being incorporated into (1).

EXPERIMENTAL

For general directions, *i.e.* counting methods, synthesis and labelling of tritium precursors, see earlier papers in this series.[12,18]

4-Benzyloxyphenyl[1-^{14}C]*acetonitrile*.—A solution of 4-benzyloxybenzyl chloride (250 mg) in dimethyl sulphoxide (5 ml) was added dropwise to a stirred suspension of potassium cyanide (activity 4.44×10^8 disint. min^{-1} + 100 mg inactive potassium cyanide) in dry dimethyl sulphoxide (5 ml). The stirring was continued overnight and the resulting mixture diluted with water (10 ml) and extracted with ether (4 × 25 ml). The combined organic layer was washed with water (25 ml), dried (Na_2SO_4), concentrated, and chromatographed over a column of neutral alumina (4.0 g) to furnish 4-benzyloxyphenyl[1-^{14}C]acetonitrile (200 mg) as an oil (lit.,[19] oil) (specific activity 9.32×10^5 disint. min^{-1} mg^{-1}).

4-Benzyloxyphenyl[1-^{14}C]*acetic Acid*.—A mixture of the preceding nitrile (200 mg), ethane-1,2-diol (5 ml), potassium hydroxide (100 mg), and water (2 ml) was refluxed for 18 h. The resulting mixture was diluted with water (5 ml) and extracted with ether (4 × 25 ml). The combined organic layer was washed with water (2 × 25 ml), dried (Na_2SO_4), and concentrated to furnish 4-benzyloxyphenyl[1-^{14}C]acetic acid (155 mg), m.p. 120—121° (lit.,[7] 120—121°) (specific activity 8.43×10^5 disint. min^{-1} mg^{-1}).

4-Benzyloxyphenyl[1-^{14}C]*acetyl Chloride*.—A mixture of the foregoing acid (155 mg) and thionyl chloride (0.5 ml) was stirred for 2 h at room temperature followed by 1 h at 40—50 °C and finally refluxed on a steam-bath for a further 1 h. Excess of thionyl chloride was removed under reduced pressure to afford 4-benzyloxyphenyl[1-^{14}C]acetyl chloride (150 mg) as an oil (lit.,[20] oil) (specific activity 7.76×10^5 disint. min^{-1} mg^{-1}).

N-(3,4-*Dibenzyloxyphenethyl*)-4-*benzyloxyphenyl*[1-^{14}C]-*acetamide*.—A solution of the preceding chloride (150 mg) in benzene (2 ml) was added to a mixture of 3,4-dibenzyloxyphenethylamine (165 mg), benzene (3 ml), and 4N-sodium hydroxide (1 ml). The reaction mixture was stirred for 2 h and the organic layer was separated, washed with N-hydrochloric acid (2 ml) and water (4 × 5 ml), dried (Na_2SO_4), and concentrated. The residue was chromatographed over neutral alumina (6.0 g) to furnish *N*-(3,4-dibenzyloxyphenethyl)-4-benzyloxyphenyl[1-^{14}C]acetamide (220 mg), m.p. 125° (lit.,[1] 125°) (specific activity 3.52×10^5 disint. min^{-1} mg^{-1}).

[1-^{14}C]-6,7-*Dibenzyloxy*-1-(4-*benzyloxybenzyl*)-3,4-*dihydroisoquinoline*.—To a solution of the foregoing acetamide (220 mg) in dry acetonitrile (5 ml) was added freshly distilled phosphorus oxychloride (1 ml) at 98 °C. The reaction mixture was cooled and stirred for 20 h at room temperature after which time the excess of phosphorus oxychloride and the solvent were removed under reduced pressure. The residue was triturated with dry ether and the product was crystallised from methanol–ether to afford [1-^{14}C]-6,7-dibenzyloxy-1-(4-benzyloxybenzyl)-3,4-dihydroisoquinoline hydrochloride (180 mg), m.p. 168° (lit.,[1] m.p. 167—168°) (specific activity 3.52×10^5 disint. min^{-1} mg^{-1}).

[1-^{14}C]-1,2-*Didehydronorcoclaurine* (6) *Hydrochloride*.—A solution of the preceding isoquinoline (180 mg) in methanol (5.0 ml) and 12N-hydrochloric acid (2.5 ml) was refluxed for 2 h. It was concentrated *in vacuo* and the residue was crystallised from methanol–ether to furnish [1-^{14}C]-1,2-didehydronorcoclaurine (6) hydrochloride (85 mg) (Found: C, 62.6; H, 5.45; N, 4.45. $C_{16}H_{16}ClNO_3$ requires C, 62.84; H, 5.28; N, 4.58%) (specific activity 6.65×10^5 disint. min^{-1} mg^{-1}).

Feeding Experiments.—The labelled precursors, *e.g.* tyrosine, tyramine, dopa, and dopamine, were fed as their hydrochlorides in aqueous solution while norcoclaurine-1-carboxylic acid, 1,2-didehydronorcoclaurine, and norcoclaurine hydrochlorides and 4-hydroxyphenylpyruvic acid were fed in aqueous dimethyl sulphoxide by stem-cut method to young *A. reticulata* shoots. The plants were left for 5—6 days for metabolism and worked up for coclaurine.

Isolation of Coclaurine.—The stems and leaves (typically 75 g wet weight) were macerated in ethanol (500 ml) with inactive coclaurine (80 mg) and left overnight. The ethanolic extract was decanted and the residue was percolated with fresh ethanol (5 × 200 ml). The combined ethanolic extract was concentrated under reduced pressure. The green viscous mass so obtained was extracted with aqueous N-acetic acid (4 × 2 ml). The acidic layer was washed with ethyl acetate (4 × 10 ml) and then basified with 6N-sodium bicarbonate (8 ml). The liberated bases were extracted with ethanol–chloroform (1 : 3, 4 × 25 ml). The combined organic layer was washed with water (2 × 15

ml), dried (Na_2SO_4), and the solvent removed under reduced pressure to afford coclaurine (50 mg) which was converted into its hydrochloride, m.p. 256—257° (lit.,[21] 255—256°), and crystallised three times from methanol–ether to constant activity. The radiopurity of the biosynthetic coclaurine was checked by reverse dilution technique and conversion to *NOO*-trimethylcoclaurine.

Degradation of Coclaurine.—(±)-[2-^{14}C]*Tyrosine feeding.* The labelled (±)-coclaurine (90 mg) (specific activity 6.62×10^2 disint. min^{-1} mg^{-1}; molar activity 1.88×10^5 disint. min^{-1} $mmol^{-1}$) in methanol (4 ml) was treated with an excess of ethereal diazomethane to give *OO*-dimethylcoclaurine which was then treated with formaldehyde (2 ml; 37—41%) and formic acid (2 ml; 98%) to afford *NOO*-trimethylcoclaurine (75 mg), m.p. 61° (lit.,[22] 61—62°) (specific activity 5.44×10^2 disint. min^{-1} mg^{-1}; molar activity 1.88×10^5 disint. min^{-1} $mmol^{-1}$).

A solution of (3) (75 mg) in methanol (5 ml) was refluxed with methyl iodide (1 ml) to give *NOO*-trimethylcoclaurine methiodide (74.5 mg) which was taken up in methanol and passed through freshly regenerated Amberlite IR-410 resin (2.0 g) to afford *NOO*-trimethylcoclaurine methohydroxide. The solution was concentrated to 5 ml, refluxed for 8 h with potassium hydroxide (0.5 g), then cooled and extracted with ether (4 × 10 ml). The combined organic layer was washed with water, dried (Na_2SO_4), and the solvent removed to furnish a mixture of *cis*- and *trans*-1-[3,4-dimethoxy-6-(β-*NN*-dimethylamino)ethylphenyl]-2-(4-methoxyphenyl)-ethylene (7) (60 mg) This mixture in ethanol was hydrogenated over platinum oxide (30 mg) to give 1-[3,4-dimethoxy-6-(β-*NN*-dimethylamino)ethylphenyl]-2-(4-methoxyphenyl)ethane (8) (55 mg) (specific activity 5.19×10^2 disint. min^{-1} mg^{-1}; molar activity 1.88×10^5 disint. min^{-1} $mmol^{-1}$). A solution of (8) in methanol was refluxed with methyl iodide to afford the corresponding methiodide which was converted to its hydroxide form by Amberlite IR-410 resin. The methohydroxide on treatment with potassium hydroxide yielded 1-[3,4-dimethoxy-6-vinylphenyl]-2-(4-methoxyphenyl)ethane (9) (42 mg) (specific activity 6.20×10^2 disint. min^{-1} mg^{-1}; molar activity 1.86×10^5 disint. min^{-1} $mmol^{-1}$).

Ozonized oxygen was passed through a solution of compound (9) (42 mg) in ethyl acetate (5 ml) for 20 min at −60 °C. The solvent was removed under reduced pressure and water (12 ml), zinc dust (120 mg), and silver nitrate (6 mg) were added to the residue. The reaction mixture was refluxed for 20 min and steam-distilled. The distillate was collected in a solution of dimedone (120 mg) in aqueous ethanol (3 : 1; 25 ml). After storing for 1 h, it was concentrated to 10 ml and left overnight. The precipitated solid (10 mg) was filtered, washed with water, dried, and chromatographed over silica gel (1.0 g). Elution with chloroform (t.l.c.) afforded the formaldehyde dimedone which was crystallised from ethanol as needles (8 mg), m.p. 193—194° (lit.,[18] 193—194°) (specific activity 3.14×10^2 disint. min^{-1} mg^{-1}; molar activity 0.92×10^5 disint. min^{-1} $mmol^{-1}$).

(±)-*Dopa feeding.* Labelled coclaurine (120 mg) (specific activity 5.29×10^2 disint. min^{-1} mg^{-1}; molar activity 1.51×10^5 disint. min^{-1} $mmol^{-1}$) was converted into *NOO*-trimethylcoclaurine (102 mg) (specific activity 4.62×10^2 disint. min^{-1} mg^{-1}; molar activity 1.51×10^5 disint. min^{-1} $mmol^{-1}$) as above. Refluxing with methyl iodide (1.5 ml) in methanol gave *NOO*-trimethylcoclaurine methiodide (100 mg) which was subjected to Hofmann degradation to give a mixture of *cis* and *trans* olefin (7) (92.5 mg). Catalytic hydrogenation in ethanol with platinum oxide afforded the dihydro derivative (8) (88 mg) (specific activity 4.37×10^2 disint. min^{-1} mg^{-1}; molar activity 1.50×10^5 disint. min^{-1} $mmol^{-1}$).

A solution of (8) in methanol was refluxed with methyl iodide to afford the corresponding methiodide (87.5 mg) which was converted into its hydroxide form with Amberlite IR-410 resin and then treated with potassium hydroxide to yield 1-[3,4-dimethoxy-6-vinylphenyl]-2-(4-methoxyphenyl)ethane (9) (55 mg) (specific activity 4.97×10^2 disint. min^{-1} mg^{-1}; molar activity 1.48×10^5 disint. min^{-1} $mmol^{-1}$). Ozonolysis of (9) gave formaldehyde which was trapped as its dimedone derivative (12.0 mg) (specific activity 5.07×10^2 disint. min^{-1} mg^{-1}; molar activity 1.48×10^5 disint. min^{-1} $mmol^{-1}$).

Tyramine feeding. The labelled coclaurine (105 mg) (specific activity 1.93×10^4 disint. min^{-1} mg^{-1}; molar activity 5.51×10^6 disint. min^{-1} $mmol^{-1}$) was converted into *NOO*-trimethylcoclaurine (100 mg) (specific activity 4.55×10^3 disint. min^{-1} mg^{-1}; molar activity 1.49×10^5 disint. min^{-1} $mmol^{-1}$) as above and treated with methyl iodide to give *NOO*-trimethylcoclaurine methiodide (98.5 mg), which was converted to the methohydroxide with Amberlite IR-410 resin. The solution was concentrated to 5 ml and refluxed for 2 h with potassium hydroxide (0.5 g). It was then cooled and worked up as above to yield a mixture of *cis* and *trans* olefin (7). This mixture (68 mg) was oxidised with potassium permanganate (85 mg) in 50% aqueous pyridine to afford *p*-anisic acid (20 mg), m.p. 183° (specific activity 10 disint. min^{-1} mg^{-1}).

4-*Hydroxyphenylpyruvic acid feeding.* Labelled coclaurine (140 mg) (specific activity 1.56×10^3 disint. min^{-1} mg^{-1}; molar activity 4.46×10^5 disint. min^{-1} $mmol^{-1}$) was degraded by the procedure as described for tyramine feeding to furnish *p*-anisic acid (27.5 mg) (specific activity 7.37×10^2 disint. min^{-1} mg^{-1}; molar activity 1.12×10^5 disint. min^{-1} $mmol^{-1}$).

(±)-*Norcoclaurine-1-carboxylic acid feeding.* Labelled coclaurine (200 mg) (specific activity 1.26×10^2 disint. min^{-1} mg^{-1}; molar activity 3.59×10^4 disint. min^{-1} $mmol^{-1}$) in methanol (6 ml) was converted into *NOO*-trimethylcoclaurine (184.5 mg) (specific activity 1.09×10^2 disint. min^{-1} mg^{-1}; molar activity 3.58×10^4 disint. min^{-1} $mmol^{-1}$) and then to *NOO*-trimethylcoclaurine methohydroxide and a mixture of *cis* and *trans* olefin (7) (155 mg).

A solution of this mixture, on catalytic hydrogenation followed by treatment with methyl iodide, Amberlite IR-410 resin, and potassium hydroxide, successively, gave (9) (115.5 mg) (specific activity 1.18×10^2 disint. min^{-1} mg^{-1}; molar activity 3.54×10^4 disint. min^{-1} $mmol^{-1}$) which was ozonized at −60 °C to afford formaldehyde dimedone (21.5 mg), m.p. 193—194° (specific activity 1.20×10^2 disint. min^{-1} mg^{-1}; molar activity 3.53×10^4 disint. min^{-1} $mmol^{-1}$).

[7/1804 *Received*, 13*th October*, 1977]

REFERENCES

[1] D. H. R. Barton, D. S. Bhakuni, G. M. Chapman, G. W. Kirby, L. J. Haynes, and K. L. Stuart, *J. Chem. Soc.* (*C*), 1967, 1295.

[2] D. S. Bhakuni, S. Satish, H. Uprety, and R. S. Kapil, *Phytochemistry*, 1974, **13**, 2767.

[3] D. H. R. Barton, D. S. Bhakuni, G. M. Chapman, and G. W. Kirby, *J. Chem. Soc.* (*C*), 1967, 2134.

[4] D. H. R. Barton, G. W. Kirby, and A. Weichers, *Chem. Comm.*, 1966, 266.
[5] D. S. Bhakuni, V. M. Labroo, A. N. Singh, and R. S. Kapil, *J.C.S. Perkin I*, 1978, 121.
[6] D. S. Bhakuni, A. N. Singh, S. Jain, and R. S. Kapil, *J.C.S. Chem. Comm.*, 1978, 226.
[7] J. Finkelstein, *J. Amer. Chem. Soc.*, 1951, **73**, 550.
[8] T. Kametani, S. Takano, K. Masuko, and S. Kuribara, *Yakugaku Zasshi*, 1965, **85**, 166.
[9] S. Teital and A. Brossi, *J. Heterocyclic Chem.*, 1968, **5**, 825.
[10] J. Fridrichsons and A. McL. Mathieson, *Tetrahedron*, 1968, **24**, 5785.
[11] E. Winterstein and G. Trier, 'Die Alkaloids,' Borntrager, Berlin, 1910, p. 307.
[12] D. S. Bhakuni, A. N. Singh, S. Tewari, and R. S. Kapil, *J.C.S. Perkin I*, 1977, 1662.
[13] M. L. Wilson and C. J. Coscia, *J. Amer. Chem. Soc.*, 1975, **97**, 431.
[14] A. R. Battersby, R. C. F. Jones, and R. Kazlauskas, *Tetrahedron Letters*, 1975, 1873.
[1] J. R. Gear and I. D. Spenser, *Nature*, 1961, **191**, 1393; H. Rapoport, N. Levy, and F. R. Stermitz, *J. Amer. Chem. Soc.*, 1961, **83**, 4298; I. D. Spenser and J. R. Gear, *ibid.*, 1962, **84**, 1059.
[16] A. R. Battersby, J. L. McHugh, J. Staunton, and M. Todd, *Chem. Comm.*, 1971, 985.
[17] *Cf.* A. R. Battersby, R. C. F. Jones, R. Kazlauskas, C. Poupat, C. W. Thornber, S. Ruchirawat, and J. Staunton, *J.C.S. Chem. Comm.*, 1974, 773.
[18] D. S. Bhakuni, S. Tewari, and R. S. Kapil, *J.C.S. Perkin I*, 1977, 706.
[19] F. Leonard and K. Undheim, U.S.P. 152,159/1964 (*Chem. Abs.*, 1965, **62**, 565).
[20] D. H. R. Barton and G. W. Kirby, *J. Chem. Soc.*, 1962, 806.
[21] K. Kratzl and G. Billek, *Monatsh.*, 1951, **82**, 568.
[22] M. Tomita and J. Kunitomo, *J. Pharm. Soc. Japan*, 1962, **82**, 734.