

## A Sesquiterpene *ortho*-Naphthoquinone from the Root Bark of *Ulmus davidiana* Planch

Jong-Pyung Kim, Won-Gon Kim, Jong Hee Park<sup>1</sup>

Jin Jung<sup>2</sup>, Hiroyuki Koshino<sup>3</sup> and Ick-Dong Yoo\*

Korea Research Institute of Bioscience and Biotechnology, Korea Institute of Science & Technology, P. O. Box 115, Yusong, Taejeon 305-600, Korea; <sup>1</sup>College of Pharmacy, Pusan National University, Pusan 609-735, Korea; <sup>2</sup>Department of Agricultural Chemistry, Seoul National University, Suwon 441-744, Korea; and <sup>3</sup>The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama, 351-01, Japan.

**Abstract** – A sesquiterpene *ortho*-naphthoquinone was isolated from the 80% aqueous methanolic extract of root bark of *Ulmus davidiana* Planch whose stem and root bark have been used as an oriental medicine for the treatment of edema, mastitis, gastric cancer and inflammation. On the basis of spectral data obtained from UV-vis, IR, HR-EIMS and NMR spectrometry, including the pulse field gradient (PFG) HMQC and HMBC techniques, the structure of this compound was determined as 2,3-dihydro-3-hydroxy-3,6,9-trimethylnaphtho(1,8-*b.c*)pyran-7,8-dione which has been known as mansonone I. This compound has been isolated only from the heart-wood of *Mansonia altissima* Chev, a Sterculiaceae from West Africa. Thus, this compound was isolated for the second time from natural source in this study. This is the first report to present the carbon chemical shift assignment of mansonone I.

**Key words** – *Ulmus davidiana*; Ulmaceae, root bark; sesquiterpene *o*-naphthoquinone; mansonone I.

*Ulmus davidiana* Planch (Ulmaceae) is a deciduous tree which is widely distributed in Korea, Japan and China. The stem and root bark of this species have been used as an oriental medicine for the remedy of edema, mastitis, gastric cancer and inflammation.<sup>1-4)</sup> As a part of our search for new biologically active substances from the folk medicines,<sup>5-7)</sup> we investigated the chemical constituents of *Ulmus davidiana* Planch.

Up to now, two flavonoidal compounds, (+)-catechin and (+)-catechin 5-O- $\beta$ -D-

apiofuranoside,<sup>8)</sup> and three sesquiterpenes, mansonones E, F and H,<sup>9)</sup> have so far been isolated from this plant species. From the 80% aqueous methanolic extracts of the root bark of this species, a sesquiterpene *ortho*-naphthoquinone was isolated. Sesquiterpene *o*-naphthoquinones were isolated for the first time from the heart wood of West African tree *Mansonia altissima* Chev (Sterculiaceae) by Sandermann et al.<sup>10)</sup> About 40 kinds of sesquiterpene *o*-naphthoquinones including mansonones,<sup>11-17)</sup> a-zanzones,<sup>18)</sup> thespone,<sup>19)</sup> etc. have been isolated from various plant species.<sup>20)</sup> This paper deals with the isolation and struc-

\*Author for correspondence : Fax 042-860-4595

tural identification of a sesquiterpene *o*-naphthoquinone, mansonone I, using extensive NMR spectroscopies including PFG-HMQC and PFG-HMBC techniques.<sup>21-23)</sup>

## EXPERIMENTAL

**General experimental procedures**—NMR spectra were recorded on a JEOL  $\alpha$ -600 NMR spectrometer at 600.05 MHz for  $^1\text{H}$ , and 150.8 MHz for  $^{13}\text{C}$  in  $\text{CD}_3\text{OD}$  with TMS as an internal standard. Chemical shifts are expressed as  $\delta$  (ppm) from TMS and coupling constants are given in Hz. All one-bond  $^1\text{H}$ - $^{13}\text{C}$  connectivities and  $^1\text{H}$ - $^{13}\text{C}$  long range correlations were established by heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC) spectrum, respectively, using pulse field gradient (PFG) techniques recorded on a JEOL  $\alpha$ -600.05 NMR spectrometer. PFG was applied by 600.5/150.8 MHz with  $^nJ_{\text{CH}}=8.3$  Hz for HMBC and  $^1J_{\text{CH}}=145$  Hz for HMQC, using a gradient of GRAD 1=20% ( $28.8 \text{ G cm}^{-1}$ ) and GRAD 2=10% ( $14.4 \text{ G cm}^{-1}$ ). Molecular weight was determined by high resolution electron impact mass spectrum (HR-EIMS) and EIMS recorded on a JEOL JMS-HX 110A mass spectrometer and Hewlett Packard Model HP 5989A EIMS system, respectively. UV-vis spectrum was taken in MeOH on a Shimadzu UV-260 spectrophotometer. IR spectrum was obtained with a Laser Precision Analect RFX-65S FT-IR spectrometer using KBr disc. Specific rotation ( $[\alpha]_D$ ) was measured in MeOH on a Polartronic polarimeter (Universal, Schmidt+Haensch, Germany) at 22°C. Melting point was measured with MEL-TEMP II (Laboratory device) and uncorrected. Column chromatography was carried out on silica gel

(Kiesel gel 60, 70-230 mesh, Merck) and Sephadex LH-20 (25-100  $\mu\text{m}$ , Pharmacia). Thin-layer chromatography (TLC) was performed using Merck silica gel plates (Kiesel gel 60F<sub>254</sub>, 0.25 mm thick: analytical, and 0.5 mm thick: preparative) without activation. Preparative HPLC was performed on a C-18 Maxsil column (22.5 mm  $\times$  250 mm, 5  $\mu\text{m}$ , Phenomenex) using a water-methanol system as a mobile phase, and by monitoring with a Waters photodiode-array detector (190-650 nm).

**Plant material**—The root bark of *Ulmus davidiana* was collected from Mt. Wonhyo, Kyungnam Province, Korea, in May 1994. Fresh root bark was dried in a dark, well-ventilated place. The voucher specimen is deposited in the Herbarium of College of Pharmacy, Pusan National University.

**Extraction and isolation**—Air dried root bark (4 kg) was milled and extracted with 80% aqueous methanol at room temperature for 3 days (3 times). The methanolic extract was filtered and concentrated under reduced pressure. The crude product (185 g) was extracted with *n*-hexane and water. The aqueous layer was reextracted with  $\text{CHCl}_3$ , and the organic layer was concentrated under vacuum. The residue (6.4 g) was subjected to column chromatography on a silica gel (60  $\times$  200 mm) eluted with  $\text{CHCl}_3$ .  $\text{CHCl}_3$ -MeOH mixture in increasing proportion of MeOH. The fractions were collected and combined by monitoring with analytical TLC to afford four fractions in order of increasing polarity. Among them, the second fraction was rechromatographed on a silica gel (35  $\times$  100 mm) with  $\text{CHCl}_3$ -MeOH mixture of (60:1)~(5:1) followed by Sephadex LH-20 (30  $\times$  850 mm) with MeOH. Preparative silica gel TLC and C-18 HPLC

(58% aqueous MeOH, flow rate 7.0 ml/min, RT 27.5 min) of the yellow fractions of which the Rf 0.32 on analytical silica gel TLC (CHCl<sub>3</sub>:MeOH=25:1) afforded 14.5 mg of pure compound 1.

**Compound 1**—Orange needles, mp 215°C:  $[\alpha]_D^{22} + 114^\circ$  (c, 1.24, in MeOH); UV-vis,  $\lambda_{\max}$  nm (MeOH) : 220 (log  $\epsilon$  3.85), 264 (log  $\epsilon$  4.10), 373 (log  $\epsilon$  2.96), 448 (log  $\epsilon$  3.01); IR  $\lambda_{\max}$  cm<sup>-1</sup> (KBr): 3435 (OH), 1685 (C=O), 1630 (olefinic C=C), 1610 and 1575 (aromatic C=C): HR-EIMS  $m/z$  258.0898 (C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> requires 258.0892): EIMS  $m/z$  (rel. int.): 260[M+2]<sup>+</sup>(9), 258[M]<sup>+</sup>(14.3), 243[M-Me]<sup>+</sup>(11.0), 230[M-CO]<sup>+</sup>(86.5), 212[M-CO-H<sub>2</sub>O]<sup>+</sup>(18.2) 201[M-CO-CO-H](100), 187[M-CO-CO-Me](18): <sup>1</sup>H-NMR, (600.05 MHz, CD<sub>3</sub>OD)  $\delta$ : 1.56 (3H, s, 3-Me), 1.92 (3H, s, 9-Me), 2.63 (3H, s, 6-Me), 4.24 (1H, d,  $J=10.8$ Hz, H-2a), 4.30 (1H, d,  $J=10.8$ Hz, H-2b), 7.44 (1H, d,  $J=7.8$ Hz, H-5), 7.82 (1H, d,  $J=7.8$ Hz, H-4), <sup>13</sup>C-NMR (150.8 MHz, CD<sub>3</sub>OD): Table 1.

## RESULTS AND DISCUSSION

Compound 1, yellow in methanol solution, was readily soluble in MeOH and DMSO but insoluble in *n*-hexane, CHCl<sub>3</sub>, EtOAc and water. It showed molecular ion peak ([M]<sup>+</sup>) at  $m/z$  258.0898 in the HR-EIMS spectrum, which corresponds to the empirical formula of C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> (calcd., 258.0892). Its UV-vis spectrum in MeOH was almost the same as that of mansonone E and tetrahydrobiflorin whose maximum absorptions are 265, 370 and 450 nm,<sup>24)</sup> suggesting that it has the same chromophoric group as 2,3-dihydro-1-oxa-phenalenequinone-7,8. The quinoid nature of this compound was indicated by the disappearance of the color from a methanolic solution on the addition

of sodium dithionite, and its exhibition of carbonyl absorptions in the IR spectrum at 1685 cm<sup>-1</sup>. In particular, it showed the *ortho*-naphthoquinone nature in the EIMS spectrum showing the relatively intense [M+2]<sup>+</sup> ion peak at  $m/z$  260, which is the characteristic of *ortho*-naphthoquinone but not that of *para*-naphthoquinone.<sup>25)</sup> The presence of a hydroxyl group was established by the IR absorption at 3435 cm<sup>-1</sup> and the mass fragment ion at  $m/z$  212 which was considered as [M-CO-H<sub>2</sub>O]<sup>+</sup> fragment ion. There was no bathochromic shift of UV-vis spectrum on adding NaOH, suggesting that this hydroxyl group is not phenolic. <sup>1</sup>H NMR spectrum showed seven signals attributed to the protons of three methyls ( $\delta$  1.56, 1.92 and 2.63), two aromatic protons ( $\delta$  7.44 and 7.82) and two methylene protons ( $\delta$  4.24 and 4.30). Each methyl proton signals was appeared as a singlet and the aromatic protons were mutually *ortho*-coupled with a coupling constant of  $J=7.8$  Hz. The strongly deshielded aromatic methyl signal appeared as a singlet at  $\delta$  2.63 indicated its *peri* position to

**Table I.** <sup>13</sup>C NMR data for compound 1(150.8 MHz, CD<sub>3</sub>, OD)

Carbon No.	$\delta$ (ppm)
2	76.0
3	67.1
3a	140.9
4	136.7
5	131.7
6	144.9
6a	127.9
7	182.9
8	181.2
9	117.4
9a	164.8
9b	127.1
3-Me	26.0
6-Me	22.8
9-Me	7.8

the quinone carbonyl. The methylene protons were appeared as doublets ( $J=10.7$  Hz) due to their geminal couplings.

The  $^{13}\text{C}$  NMR spectrum of compound 1 showed fifteen carbons having two carbonyl carbons of quinone resonating at  $\delta$  182.9 (C-7) and 181.2 (C-8). By the aid of PFG-HMQC spectrum (Fig. 1), the carbons at  $\delta$  7.8 (9-Me), 26.0 (3-Me) and 22.8 (6-Me) were assigned as the methyl carbons bonded with the methyl protons at  $\delta$  1.92, 1.56 and 2.63, respectively. And it was revealed that the carbon at  $\delta$  76.0 (C-2) is an oxymethylene carbon and the carbons at  $\delta$  136.7 and 131.7 are aromatic carbons bond-

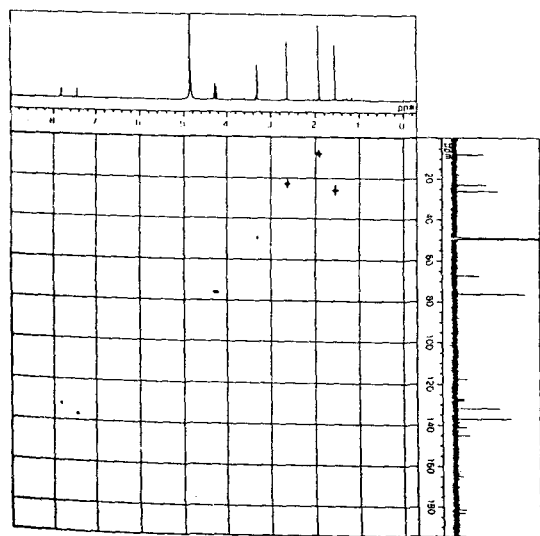
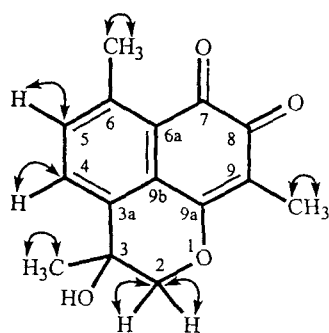


Fig. 1.  $^1\text{H}$ - $^{13}\text{C}$  correlations and PFG-HMQC spectrum of compound 1.

ed with the aromatic protons at  $\delta$  7.82 and 7.44, respectively. All other carbons were known to be quaternary carbons. Among them, the carbon at  $\delta$  164.8 was considered as an  $sp^2$  quaternary carbon which is directly linked to an oxygen atom. The carbon at  $\delta$  67.1 was considered as a hydroxylated carbon to which the non-phenolic hydroxyl group is attached.

The complete structure of the compound was determined by the  $^1\text{H}$ - $^{13}\text{C}$  long range correlations in the PFG-HMBC spectrum (Fig. 2). The methyl protons at  $\delta$  1.56 (3-Me) showed three correlations within the heterocyclic ring due to the  $^2J$  coupling with C-3 ( $\delta$  67.1) and  $^3J$  couplings with C-2 ( $\delta$  76.0) and C-3a ( $\delta$  140.9). The olefinic methine protons at  $\delta$  4.24 (H-2a) and 4.30

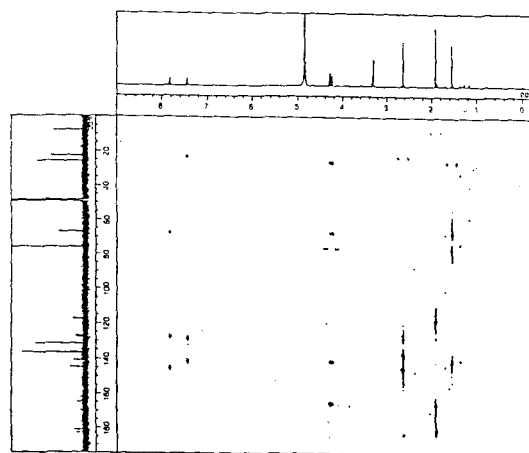
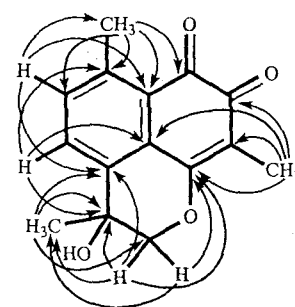


Fig. 2.  $^1\text{H}$ - $^{13}\text{C}$  long range correlations and PFG-HMBC spectrum of compound 1.

(H-2b) showed the  $^1\text{H}$ - $^{13}\text{C}$  long range correlations to C-3a, C-9a ( $\delta$  164.8) and methyl carbon at  $\delta$  26.0 (3-Me). These observations indicated the presence of an ether bond between C-2 and C-9a. The aromatic proton at  $\delta$  7.82 (H-4) showed the long range correlations to C-6 ( $\delta$  144.9), C-9b ( $\delta$  127.1) and C-3 ( $\delta$  67.1). Another *ortho*-coupled aromatic proton at  $\delta$  7.44 (H-5) was coupled to the quaternary carbons at  $\delta$  140.9 (C-3a) and  $\delta$  127.9 (C-6a), and to the methyl carbon at  $\delta$  22.8 (6-Me). From the strongly deshielded aromatic methyl protons at  $\delta$  2.63 (6-Me), the long range couplings to C-5 ( $\delta$  131.7), C-6, C-6a and C-7 ( $\delta$  182.9) were observed. Among them, the  $^4J$  coupling with C-7 proved the *peri* relationship of 6-Me to this carbonyl carbon. The remaining methyl protons at  $\delta$  1.92 displayed long range correlations to C-9a, C-9b and the carbonyl carbon at 181.2 (C-8). From these observations, the chemical shift assignments of four quaternary carbons, C-3a, C-6a, C-9a and C-9b, were established. Although the connectivity between two carbonyl carbons (C-7 and C-8) could not be proved, its *o*-quinone structure had been suggested by the physico-chemical properties. The non-phenolic hydroxyl group was attached to C-3 ( $\delta$  67.1) on the heterocyclic ring. Thus, this compound is hydroxylated mansonone E whose H-3 is substituted by a hydroxyl group. All of these observations led to the conclusion that the structure of this compound is 2,3-dihydro-3-hydroxy-3,6,9-trimethylnaphtho(1,8-*b,c*)pyran-7,8-dione, which has been known as mansonone I.<sup>14)</sup>

Mansonones have been isolated from several plant species.<sup>13,18,19,26,27)</sup> In the sapwood of *Ulmus americana* and other elm species, the accumulation of mansonones E and F

in response to the infection by *Cercotycystis ulmi* or other stresses have been observed.<sup>28-30)</sup> However, mansonone I has never been isolated from *Ulmus* species. It has been reported only from the *Mansonia altissima* Chev.<sup>14)</sup> Thus, mansonone I was isolated for the second time from natural source in this study.

### LITERATURE CITED

1. Cho, M. Y. (1989) Colored Illustrations of Plants of Korea, 107-109. Academy Press, Seoul, Korea.
2. Lee, C. B. (1985) Korea Encyclopedia of Plants, 280-281. Hyangmoonsa, Seoul, Korea.
3. Shanghai Science and Technological Publisher (1985) The Dictionary of Chinese Drugs, Vol. 4, 2771. Shougakukan, Tokyo.
4. Lee, S. J. (1966) Korean Folk Medicine, Monographs Series No. 3, 39. Publishing center of Seoul National University, Seoul, Korea.
5. Lee, I. K., Kim, C. J., Song, K. S., Kim, H. M. and Yoo, I. D. (1996) Two more benzylated dihydroflavonols from *Cudrania triscuspidata*. *J. Natural Products* 58: 1614-1617.
6. Lee, I. K., Kim, C. J., Song, K. S. and Yoo, I. D. (1996) Cytotoxic benzyl dihydroflavonols from *Cudrania triscuspidata*. *Phytochemistry* 41: 213-216.
7. Lee, I. K., Kim, C. J., Song, K. S. and Yoo, I. D. (1994) Cytotoxic and antioxidative flavonoids from the stem bark of *Cudrania triscuspidata*. *Agr. Chem. Biotech.* 37: 105-109.
8. Son, B. H., Park, J. H. and Zee, O. P. (1989) Catechin glycoside from *Ulmus davidiana*. *Arch. Pharm. Res.* 12: 219-222.
9. Kim, J. P., Kim, W. G., Koshino, H., Park, J. H., Jung, J. and Yoo, I. D. (1996) Isolation and identification of sesquiterpene *o*-naphthoquinones, mansonones E, F and H, from the root bark of *Ulmus davidiana* Planch. *Agr. Chem. Biotech.* 39: 89-94.
10. Sandermann, W. and Dietrichs, H. H. (1959) Chemical investigations of tropical woods-part III. On the extractives of *Mansonia altissima* and their injurious effect on health. *Holz. als*

- Roh- und Werkst* 3: 88-97.
11. Marini-Bett lo, G. B. M., Casinovi, C. G. and Galeffi, C. (1965) A new class of quinones: Sesquiterpenoid quinones of *Mansonia altissima* Chev. *Tetrahedron Letters* 52: 4857-4864.
  12. Tanaka, N., Yasue, M. and Imamura, H. (1966) The quinoid pigments of *Mansonia altissima* wood. *Tetrahedron Letters* 24: 2767-2773.
  13. Chen, C. M., Chen, Z. T. and Hong, Y. L. (1990) A mansonone from *Helicters angustifolia*. *Phytochemistry* 29: 980-982.
  14. Shimada, K., Yasue, M. and Imamura, H. (1967) The structure of Mansonone I, a new pigment from *Mansonia altissima*. *J. Japan Wood Res. Soc.* 13: 126.
  15. Marini-Bettlò, G. B., Casinovi, C. G., Galeffi, C. and Monache, F. D. (1966) Sui chinoni sesquiterpenici della *Mansonia altissima* Chev. *Ann. Ist. Super. Sanità* 2: 327-341.
  16. Galeffi, C., Casinovi, C. G., Miranda delle Monache, E. and Marini Bett lo, G. B. (1968) Chinoni sesquiterpenici della *Mansonia altissima* Chev. ~Nota II. *Ann. Ist Super. Sanità*: 4305-316.
  17. Galeffi, C., Miranda delle Monache, E., Casinovi, C. G. and Marini Bettlò, G. B. (1969) A new quinone from the heartwood of *Mansonia altissima* Chev: mansonone L. *Tetrahedron Letters* 40: 3583-3584.
  18. Letcher, Roy M. and Shirley, I. M. (1992) *o*-Naphthoquinones from the heartwood of *Azanza garkeana*. *Phytochemistry* 31: 4171-4172.
  19. Neelakantan, S., Rajagopalan, V. and Raman, P. V. (1983) Thespesone and thespone, two new mansonones of heartwood of *Thespesia populnea* Sol. ex Corr. (Fam. Malvaceae). *Indian J. Chem.* 22B: 95-96.
  20. Thomson, R. H. (1987) Naturally Occurring Quinones III-recent advances, 127-343. Chapman and Hall, New York, USA.
  21. Hurd, R. E. and John, B. K. (1991) Gradient-enhanced proton detected heteronuclear multiple-quantum coherence spectroscopy. *J. Magn. Reson.* 91: 648-653.
  22. Tyburn, J. M., Brereton, I. M. and Doddrell, D. M. (1992) Coherence selection in gradient-enhanced heteronuclear correlation spectroscopy. *J. Magn. Reson.* 97: 305-312.
  23. Willker, W., Leibfritz, D., Kerssebaum, R. and Bermel, W. (1993) Gradient selection in inverse heteronuclear correlation spectroscopy. *J. Magn. Reson. Chem.* 31: 287-292.
  24. Comin, J., Concalves de Lima, O. G., Grant, H. N., Jackman, L. M., Schierlein, W. K. and Prelog, V. (1963) ber die Konstitution des Biflorins, eines *o*-Chinons der Diterpen-Reihe. *Helv. Chim. Acta.* 46:409-415.
  25. Oliver, R. W. A. and Rashman, R. M. (1971) Mass spectrometry of quinones. Part II. A study of the distinguishing features found in the mass spectra of 1,2- and 1,4-naphthaquinones. *J. Chem. Soc. (B)*: 341-344.
  26. Ali, S., Singh, P. and Thomson, R. H. (1980) Naturally occurring quinones. Part 28. Sesquiterpenoid quinones and related compounds from *Hibiscus tiliaceus*. *J. C. S. Perkin Trans* 1: 257-259.
  27. Ogihara, K., Zhao, J., Higa, M. and Yogi, S. (1992) Studies on the constituents of *Aristolochia liukiuensis* II. *Bull. Coll. Sci. Univ. Ryukus* 54: 17-28.
  28. Overeem, J. C. and Elgersma, D. M. (1970) Accumulation of mansonones E and F in *Ulmus hollandica* infected with *Ceratocystis ulmi*. *Phytochemistry* 9: 1949-1952.
  29. Elgersma, D. M. and Overeem, J. C. (1971) The relation of mansonones to resistance against Dutch elm disease and their accumulation, as induced by several agents. *Neth. J. Plant Pathol.* 77: 168-174.
  30. Dumas, M. T., Strunz, G. M., Hubbes, M. and Jeng, R. S. (1983) Isolation and identification of six mansonones from *Ulmus americana* infected with *Ceratocystis ulmi*. *Experientia* 39: 1089-1091.

(Received 9 February 1996)