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Use of fluorine-containing stationary phases for the separation of an alkyl bromide from its hydrocarbon analog by high-performance liquid chromatography

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Abstract

Radiolabelled Sch 13835, an inhibitor of platelet derived growth factor, was prepared by catalytic hydrogenolysis of a benzyl bromide precursor with tritium gas. Regular and deactivated reversed-phase HPLC stationary phases gave poor peak shapes and little resolution of Sch 13835 and the benzyl bromide precursor. Fluorodecyl and fluoroether stationary phases in the analytical reversed-phase mode gave baseline separation using organic–aqueous (no buffers) mobile phases. Elution orders were reversed on either phase by a change in the organic component from methanol to acetonitrile. The product is unstable in aqueous (or other protic) solvents, forming a ring-opened acid. Conditions were developed to successfully purify the compound by reversed-phase HPLC with minimal decomposition. A second analytical HPLC assay was developed using normal-phase solvents on a fluoroether stationary phase.

Keywords: Stationary phases, LC; Fluorine-containing stationary phases; Growth factor inhibitors; Alkyl bromides

1. Introduction

Sch 13835 is a platelet derived growth factor (PDGF) inhibitor under evaluation at Schering-Plough Research Institute. [^{3}H]Sch 13835 was synthesized as shown in Scheme 1 for use in drug metabolism studies.

Our goal was to develop a preparative method to purify [^{3}H]Sch 13835 and to develop analytical systems for analysis of the radiolabelled compound.

For analytical assays of radiolabelled compounds, two or more chromatography systems as different from each other as possible, e.g. reversed and normal phase modes, are desired.

For preparative work a simple (no buffers) aqueous–organic mobile phase is preferable for ease of recovery of purified product. Because of the high specific activity and consequently low mass of the product, the preparative purification was done on an analytical-size column.

2. Experimental

2.1. Reagents and chemicals

All solvents were HPLC grade and were obtained from Fisher Scientific (Fair Lawn, NJ, USA). The injection solutions were 1 mg ml^{-1} in acetonitrile. Injection size was 10 ml=10 μg. Mobile phases were mixtures of water and either methanol or acetonitrile. No buffers or other additives were used.

The liquid scintillation cocktail used in the radioactivity detector was FLO-SCINT III (Packard

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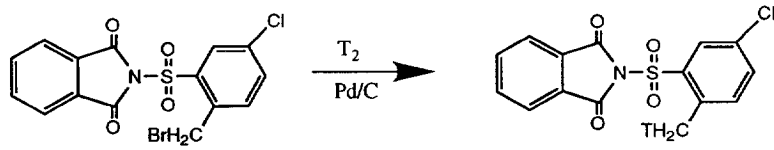


Sch 13929 ^{3}H-Sch 13835 (1)

Scheme 1.

Instruments, Meriden, CT, USA) at a flow-rate twice that of the HPLC mobile phase.

Tritium gas reductions of Sch 13929 were performed by Amersham Corporation. After removal of the catalyst and labile tritium, the crude product was shipped to us.

2.2. Equipment

The solvent and sample delivery systems (Waters, Milford, MA, USA) consisted of Models 6000 and 510 pumps and a Model 712 WISP autosampler. The UV detection system (Waters) was a Model 484 Tunable Absorbance Detector set at 240 nm. The data processing system (Waters) was a DEC Pro 380 with Waters 840 software. Data acquisition was at one point per second, with a peak width of 20 s and a peak detection threshold of 25 mV.

The radiochemical detection system (Packard Instruments) was a Radiomatic Flow-One Model CT or Model A-250. The time constant was 6 s. The tritium window was 1-40 keV for the Model CT and 0-20 keV for the Model A-250.

2.3. Columns

The Spherisorb C_8 column, 100 mm×4.6 mm I.D., was from Phase Separations (Norwalk, CT, USA). The Ultracarb ODS 30 column, 100 mm×4.6 mm I.D., was from Phenomenex (Torrance, CA, USA). The 300 mm×4.6 mm I.D. Chromegabond FD and Chromegabond FE fluorocarbon columns were from E.S. Industries (Berlin, NJ, USA).

3. Results and discussion

3.1. Separation of Sch 13835 and Sch 13929

Stationary phases were evaluated using simple aqueous–organic mobile phase mixtures, i.e. no additives were used. A separation was initially attempted on a conventional reversed-phase column, Spherisorb C_8. The stationary phase [1] was 80 Å, 5 μm spherical silica monomerically bonded with octyl ligands and then endcapped. Pore volume was 0.5 ml g^{-1} and surface area was 220 $m^2 g^{-1}$. Carbon load was 6% and bonded phase coverage was 2.51 μmol m^{2-1}. The "best" separation obtained is shown in Fig. 1a. Mobile phase was CH_3CN–water (70:30) at 1 ml min^{-1}. Peak shape and resolution were very poor. Access of solute to the free silanols of the stationary phase leads to tailing. The relatively low percentage carbon load and bonded phase coverage led to significant tailing.

Next was tried an Ultracarb ODS 30 column. The stationary phase [2] was 60 Å, 5 μm spherical silica monomerically bonded with octadecyl ligands and then endcapped. The surface area was 550 $m^2 g^{-1}$, carbon load was 31% and bonded phase coverage was 4.06 μmol m^{2-1}. Fig. 1b shows the optimum separation obtained from this ultra high coverage phase. Mobile phase was CH_3OH–water (70:30) at 1 ml min^{-1}. Peak shape was much improved, but resolution was still poor.

We then evaluated fluorocarbon stationary phase columns. The structures of the phases [3] are as shown in Scheme 2.

For each phase the fluorine load was 8.5%. Fluoroalkyl and fluorophenyl-bonded stationary phases have been found useful in reversed-phase HPLC [4–7,10]. In general they are less retentive than hydrocarbon phases. They have unique selectivity for halogenated solutes but also for solutes with various other polar groups. They can be used in both reversed- and normal-phase modes [8].

Fig. 1c and d show the optimal results using a Chromegabond FD column, [c: CH_3CN–water (50:50) at 1.5 ml min^{-1}, d: CH_3OH–water (60:40) at 1 ml min^{-1}]. Fig. 1e and f show the optimal

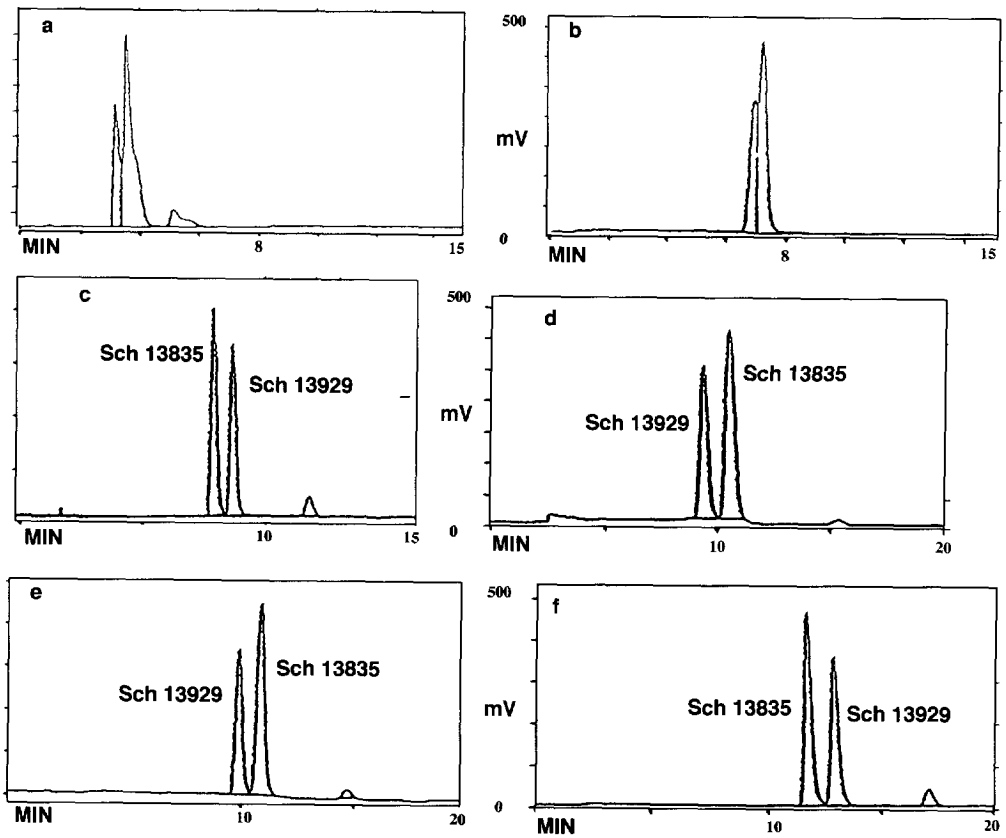


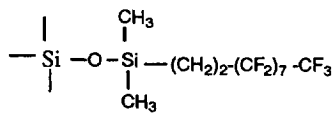
Fig. 1. UV (240 nm) chromatograms of Sch 13929–Sch 13835 reference mixture. (a) Spherisorb (b) Ultracarb (c,d) Fluorodecyl (e,f) Fluoroether. Mobile phases as in text.

results for a Chromegabond FE column, [e: CH_3CN–water (50:50) at 1 ml min^{-1}, f: CH_3OH–water (60:40) at 1 ml min^{-1}]. Peak identities were confirmed by injection of the single components.

Both columns separated the pair, along with an impurity, with good peak shape and resolution. Both columns gave peak reversals on changing the organic component of the mobile phase, i.e., Sch 13835 eluted before Sch 13929 in the acetonitrile mobile phase but after it in the methanol mobile phase.

3.2. Analysis of first radiolabelled batch

One hundred mCi of [^{3}H]Sch 13835, in ethanol, was received from Amersham Corporation. Specific



heptadecafluorodecyldimethylsilane

Chromegabond FD

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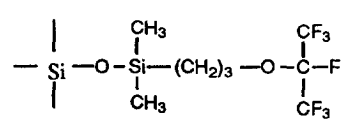


heptafluoroisopropoxypropyldimethylsilane

Chromegabond FE

Scheme 2.

activity was 4 mCi ml^{-1} and mass concentration was estimated as 140 μg ml^{-1}.

The batch was analyzed using the system described in Fig. 1f, i.e., methanol–water (60:40) on an FE column. Fig. 2a and b show the radio- and UV-chromatograms obtained. The [^{3}H]Sch 13835 peak eluted at about 10 min and represented about 25% of the total radioactivity. A column wash using the strong methanol solvent (not shown) removed a further 0.4% of highly retained radioactive material.

3.3. Purification of first radiolabelled batch

Purification was studied on a second analytical FE column. Mobile phase organic component was changed from methanol to acetonitrile because of the greater solubility of Sch 13835 in acetonitrile. Gradient elution was used. To study the effect of a high mass load on the column, an injection of 1 mg of reference Sch 13835 "spiked" with 40 μCi of labelled material was made. This is known as a "carrier-added" solution. Fig. 2c and d show the radio- and UV-chromatograms obtained. Note that peak shape and resolution was very good at the 1 mg loading level.

The ethanol solvent from an aliquot (5 ml, 20 mCi, 0.7 mg) of the batch was evaporated, dissolved in 0.1 ml acetonitrile and injected. The separation was monitored by UV and fractions were collected at the beginning, middle and end of the major peak. An analytical radiochromatogram of the middle peak fraction showed a profile similar to that of the crude material. The purification process was repeated with another 5 mCi aliquot of bulk and the same results were obtained.

These results suggested that [^{3}H]Sch 13835 was not stable under the conditions used for purification. Ongoing synthetic investigations confirmed these results [9]. The compound was shown to be stable in aprotic organic and in aqueous acidic solvents and

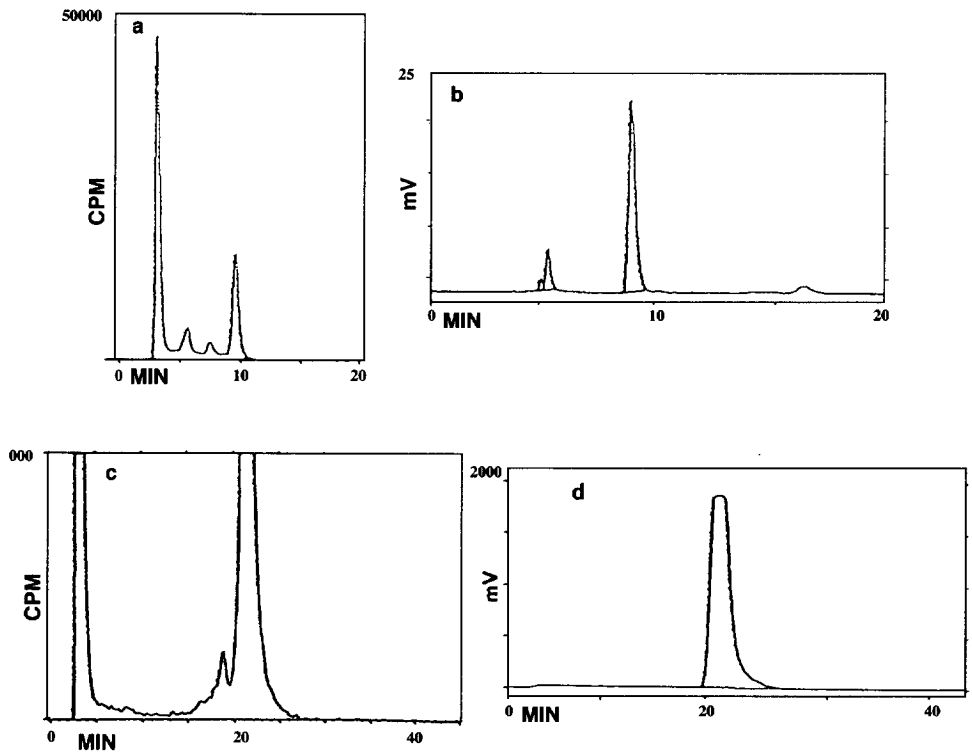
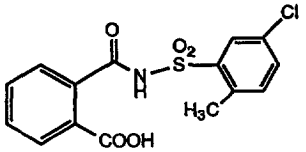


Fig. 2. Analytical (a,b) and preparative (c,d) Fluoroether radio- and UV-chromatograms of first [^{3}H]Sch 13835 batch. CH_3OH–water (60:40) at 1 ml min^{-1}.

unstable in protic media under neutral to basic conditions. A major decomposition product of Sch 13835 was the ring-opened acid shown below.



3.4. Reversed-phase analysis of second radiolabelled batch

A repeat tritiation was carried out by Amersham and the crude product was shipped as a dimethoxyethane (DME) solution at 0.1 mg ml^{-1}, 4 mCi ml^{-1}.

A stability study of this new batch was performed using 10 μg (0.7 μCi) injections in acetonitrile of carrier-added material. Fig. 3 shows the FE column radiochromatograms using 1 ml min^{-1} gradients of (a) methanol and (b) acetonitrile: 5 min isocratic 30% methanol or 25% acetonitrile, 15 min linear gradient to 100% organic, 10 min isocratic organic. Radiochemical purity was 72% for the methanol and 82% for the acetonitrile runs. Note that only one major impurity was observed and that there was a constant "bleeding" of radioactivity throughout especially the methanol chromatogram. This led us to suspect on-column decomposition.

Preparative runs were made of 50 μg of radiolabelled-spiked reference material using the acetonitrile gradient. For the first run, the collected fraction analyzed at about 85% just after peak collection and again after being stripped of solvent (Speed Vac). For the second run, the gradient curve was changed from linear (No. 6) to convex (No. 4) to minimize compound exposure to aqueous mobile phase. An aliquot of the collected peak was removed for analysis and the remaining portion was fast-frozen in liquid N_2 and later evaporated (Speed Vac) and dissolved in acetonitrile for analysis. Both solutions analyzed at about 96% radiochemical purity with only the low retention impurity observed. This impurity was proven to be the ring-opened acid shown previously by comparison to an authentic sample.

3.5. Normal-phase HPLC

For a second analytical assay system normal phase chromatography was investigated on a FD column using solvents of methylene chloride, isooctane, acetonitrile, methyl *tert.*-butyl ether and *n*-butyl chloride. Fig. 4 shows the optimal separations. Mobile phases were (a) methylene chloride–isooctane (25:75) at 1.5 ml min^{-1} and (b) *n*-butyl chloride–isooctane (60:40) at 2 ml min^{-1}. Injection solutions of the reference materials were 1 mg ml^{-1} in methylene chloride. Sch 13929 eluted before Sch 13835 in each system.

The first mobile phase system was used, with the

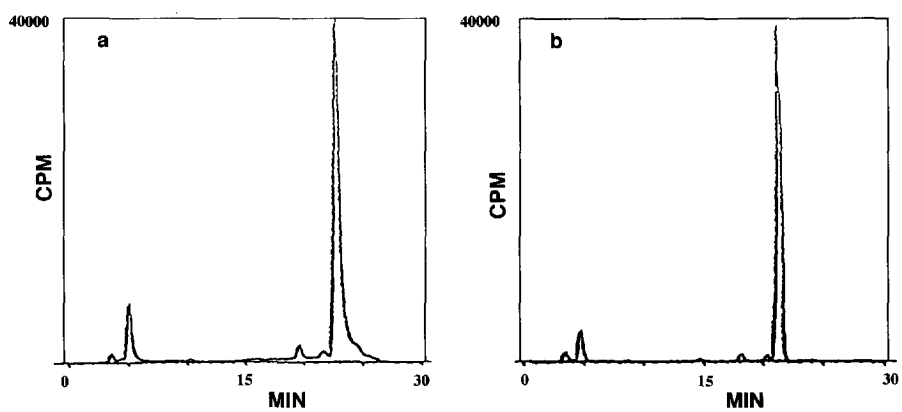


Fig. 3. Fluoroether analytical radiochromatograms of second batch of [^{3}H]Sch 13835. Gradients at 1 ml min^{-1}. (a) CH_3OH mobile phase organic component, (b) CH_3CN mobile phase organic component.

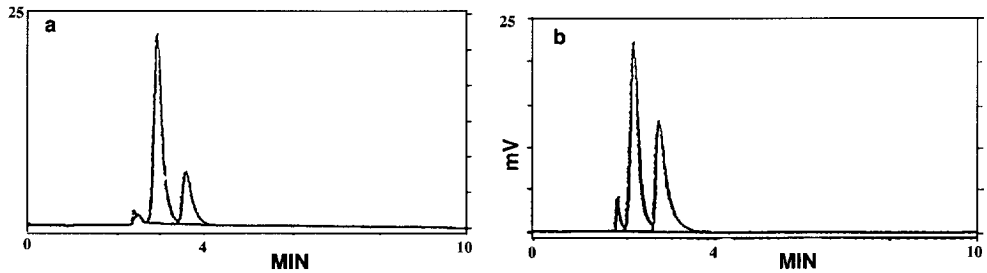


Fig. 4. UV (240 nm) chromatograms of Sch 13929–Sch 13835 reference mixture on Fluorodecyl column. (a) CH_2Cl_2–isooctane (25:75) at 1.5 ml min^{-1}. (b) *n*-butylchloride–isooctane (60:40) at 2.0 ml min^{-1}.

addition of a gradient to 100% methylene chloride, to assay the purified tracer above. Radiochemical purity was about 96%, in agreement with the reversed phase result. The gradient eluted the ring-opened degradation product (0.5%), proven first by injection of non-labelled reference compound and second by injection of a [^{3}H]Sch 13835 sample 30 min after addition of 1 *M* NaOH.

Normal- and reversed-phase studies were done using variable flow and stop flow techniques to evaluate the on-column stability of [^{3}H]Sch 13835. While the results were not clearcut, they do indicate that on-column decomposition occurred in both modes and that acetonitrile seemed to stabilize the compound in either mode.

3.6. Final purification of [^{3}H]Sch 13835

An 8 mCi (20 μg in 200 μl acetonitrile) aliquot was purified by reversed-phase HPLC using the

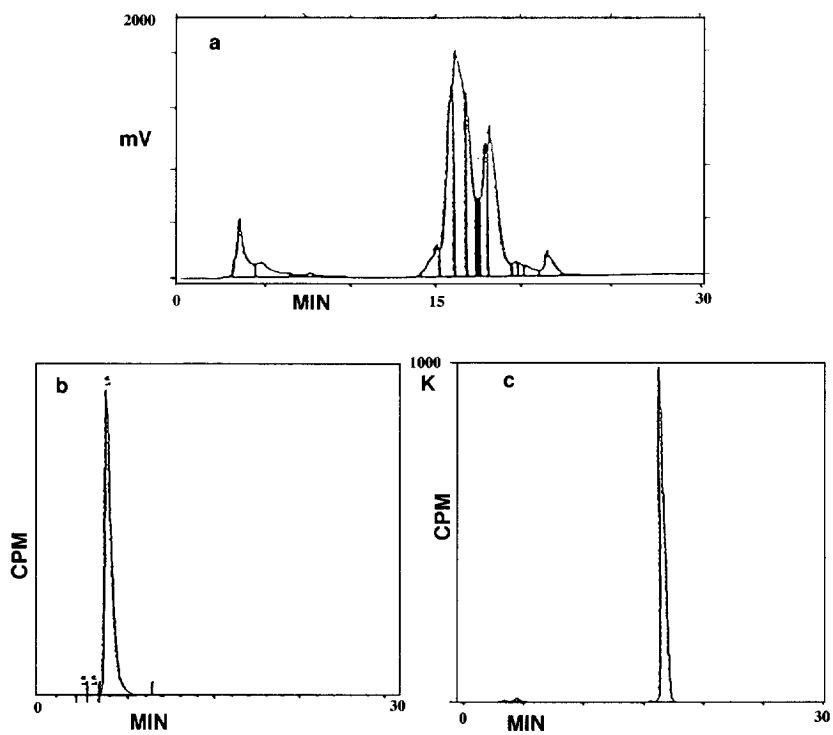


Fig. 5. Fluoroether preparative (a) UV chromatogram of crude [^{3}H]Sch 13835 and analytical (b, c) radiochromatograms of the final batch. (a, c) CH_3CN–water (25:75) to (100:0) gradient at 1 ml min^{-1}. (b) CH_3CN–CH_2Cl_2 (80:20) to (0:100) gradient at 1.5 ml min^{-1}.

following procedures to minimize radiochemical decomposition: (1) acetonitrile rather than methanol was used as the mobile phase organic component; (2) a convex gradient was used in order to minimize compound exposure to aqueous solvents; (3) fractions were collected and immediately frozen in liquid N_2. Solvent was removed under high vacuum (Speed-Vac), followed by dissolution in acetonitrile and storage at −80 °C.

Fig. 5a shows the UV chromatogram of the preparatory run. Fractions were collected as follows: fraction A: 3.3–3.9 min, fraction B: 14.2–15.2 min, fraction C: 15.2–17.5 min, fraction D: 17.5–19.3 min.

Fraction C contained the bulk of the radioactivity, 6.3 mCi. Fig. 5b and c show the analytical radiochromatograms of the normal and reversed-phase modes. The purity values were 99.3% and 96.2% respectively and the batch was suitable for drug metabolism studies.

4. Conclusion

Fluorine-containing stationary phases provided unique selectivity for the separation of an alkylbromide (Sch 13929) precursor from its reduced hydrocarbon (Sch 13835) analog. Using simple aqueous–organic mobile phases giving poor resolution and selectivity on conventional reversed-phase stationary phases both analytical and semipreparative separations were obtained. The same fluorine-containing stationary phases also gave useful normal-phase separations.

References

[1] Column Catalogue, MetaChem Technologies Inc., Torrance, CA, 1996, p. 6.
[2] Column Catalogue, Phenomenex, Torrance, CA, 1996, p. 185.
[3] P.C. Sadek and P.W. Carr, J. Chromatogr., 288 (1984) 25–41.
[4] N.D. Danielson, L.G. Beaver and J. Wangsa, J. Chromatogr., 544 (1991) 187–199.
[5] S. Yang, L.F. Resotho Kruk and M.G. Khaledi, J. Chromatogr. A, 664 (1994) 1–11.
[6] S.L. Richheimer, M.C. Kent and M.W Bernart, J. Chromatogr. A, 677 (1994) 75–80.
[7] K. Jinno and H. Nakamura, Chromatographia, 39 (1994).
[8] Column Catalogue, E.S. Industries, Berlin, NJ, 1996, p. 12.
[9] S. Rosenblum, SPRI Chemical Research Department, private communication.
[10] S.L. Richheimer et al., Anal. Chem., 64 (1992) 2323.