Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 1473–1477

Screening yeasts for the stereoselective reduction of oxoester clofibrate analogues

Maria Grazia Perrone,[a] Ernesto Santandrea,[a] Antonio Scilimati,[a,*] Christoph Syldatk[b] and Vincenzo Tortorella[a]

[a]*Dipartimento Farmaco-Chimico, Università di Bari, Via E.Orabona 4, 70125 Bari, Italy*
[b]*Universität Karlsruhe (TH), Engler-Bunte-Institut, Lehrstuhl für Technische Biologie, Engler-Bunte-Ring 1, 76131 Karlsruhe, Germany*

Received 12 January 2004; accepted 1 February 2005
Available online 16 March 2005

Abstract—Reduction of oxoesters **1b–d** and **1f,g** in the presence of different yeast strains (*Saccharomyces cerevisiae* DSM 11285, *S. cerevisiae* CBS 7336, *Cryptococcus curvatus* ATCC 20509, *Candida bombicola* ATCC 22214, *Trigonopsis variabilis* DSM 70714, *Kluyveromyces marxianus* CBS 6556) affords hydroxy esters **2b–d** and **2f,g** with diastereoisomeric excesses (de) up to >99%. Hydrolytic enzyme(s) contained in the yeasts catalyzed to some extent the hydrolysis of the oxoesters to the corresponding acids, which undergo decarboxylation followed by reduction of the carbonyl moiety.

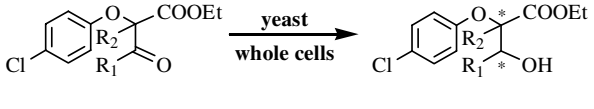
1. Introduction

Clofibrate belongs to a class of drugs used to treat hyperlipidemias, atherosclerosis and for the prevention of heart failure. Fibrates are well tolerated in the clinic, and show favourable safety profiles, even if fibrate-associated toxicity has been reported in almost every organ system.[1] The most pronounced contraindication is the liver and renal insufficiency.[2,3]

In our previous investigation, a number of racemic and optically active clofibrate analogues were synthesized and pharmacologically evaluated as peroxisome proliferator-activated receptor activators[4] and muscle tissue chloride channel affecting agents[5–7] for their therapeutic activity and toxic side effects, respectively, with the aim of discriminating the structural determinants responsible for the different activities. The configuration of clofibrate analogues having a stereogenic centre also affects their pharmacological profiles (therapeutic and adverse side effects).[8–14]

Recently, baker's yeast and a number of other yeasts, valuable tools for stereoselectively reducing prochiral ketones, have been used by us to prepare almost enantiomerically pure 2-(4-chlorophenoxy)-3-hydroxybutanoic acid, a new clofibrate analogue.[15,16]

Usually, baker's yeast constitutes the first choice catalyst to accomplish carbonyl bioreductions, because it is easy to use and does not require any specific laboratory equipment. However, we have highlighted that other microbes, such as *Kluyveromyces marxianus* CBS 6556, can also catalyze this asymmetric bioreduction, sometimes outperforming baker's yeast in terms of stereoselectivity and activity, as in the case of the reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1a** (Scheme 1) (de >99%, $ee_{(2R,3S)}$ = 97%, and de = 92%, $ee_{(2R,3S)}$ = 94% for the reaction performed in the presence of baker's yeast).[16] Herein, as a continuation of our previous



1a-g **2a-g**

	a	b	c	d	e	f	g
R_1	CH_3	C_2H_5	n-C_3H_7	i-C_3H_7	t-C_4H_9	C_6H_5	CH_3
R_2	H	H	H	H	H	H	CH_3

Scheme 1. Bioreduction of compounds **1a–g** by yeasts.

* Corresponding author. E-mail: ascilimati@farmchim.uniba.it

0957-4166/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2005.02.010

investigation aimed at preparing other optically active clofibrate analogues,[15,16] we report the results of the reduction of the 3-oxoesters **1b–g** (Scheme 1) carried out in the presence of some microbes.

2. Results and discussion

The reductive properties of a number of yeast strains (*Saccharomyces cerevisiae* DSM 11285, *S. cerevisiae* CBS 7336, *Cryptococcus curvatus* ATCC 20509, *Candida bombicola* ATCC 22214, *Trigonopsis variabilis* DSM 70714, *K. marxianus* CBS 6556) in the presence of compounds **1b–g** have been evaluated in an attempt to develop a diastereo- and enantioselective method to transform 3-oxoesters into 3-hydroxyesters.

Compound **1a** was diastereo- and enantioselectively converted into **2a** (as said in Section 1) by baker's yeast mediated reduction.[15,16] The results of the reduction of **1a–g** performed in the presence of the above mentioned yeast strains are summarized in Table 1.

All the substrates were successfully reduced with the exception of **1e**. No product isolation and characterization was possible for **2e**: in all the conditions used, the conversion was lower than 1%. Reported data (Table 1) show that each yeast behaves differently for each substrate.

As far as **1b** is concerned, after 5 reaction days, the conversion into **2b** mediated by both strains of *S. cerevisiae* (conv. in **2b** = 84%), is comparable to that obtained carrying out the reduction in the presence of baker's yeast (conv. in **2b** = 88%). On the contrary, no diastereoselectivity was observed with both *S. cerevisiae*, whereas a de = 50% was seen in the case of baker's yeast. A higher amount of the hydroxyester **2b** (95%) and de = 40% were found in the case of *C. bombicola*. *T. variabilis* gave 83% of **2b** and de = 60% after 4 reaction days. It is noteworthy that the reduction of **1b** mediated by *K. marxianus* was quite unsatisfactory, when compared to the behaviour of the same yeast in the reduction of **1a**.[16] In particular, a moderate increase in the side chain length (a methyl in **1a**, an ethyl in **1b**) caused a substantial lowering of both **2b** formation (69%) and de (54%), together with a remarkable increase in the reaction time (5 days vs 8 h). When *K. marxianus* was used to mediate the reduction of **1c**, in which the side chain is an *n*-propyl, 31% of **2c** formed. For the reduction of **1c** reaction times were always higher (4–11 days) than those with baker's yeast (**1c** quantitatively converted within 1.5 days) while **2c** percentages were always lower, ranging between 0% and 69%. Comparable diastereoisomeric excesses were obtained with both strains of *S. cerevisiae* (de = 60% and 66%), although they were lower than baker's yeast, which gave a de (89%) similar to that of *T. variabilis* (de = 86%). A substantial de (>98%) was obtained in the presence of *C. bombicola*.

Results of the bioreduction of **1d** depended on the yeast strain used, and in any case represent an improvement of the data obtained for the reaction performed in the presence of baker's yeast. In fact, **1d** was quantitatively converted into the product from both strains of *S. cerevisiae* within 1 day, while 88% of product was obtained with *C. bombicola* after 5 incubation days. These results were better than 43% conversion observed within 4 days

Table 1. Results of the whole cell-mediated reduction of **1a–d** and **1f,g**

Compd	Product/reaction time/de	*Saccharomyces cerevisiae* DSM 11285	*Saccharomyces cerevisiae* CBS 7336	*Candida bombicola* ATCC 22214	*Cryptococcus curvatus* ATCC 20509	*Trigonopsis variabilis* DSM 70714	*Kluyveromyces marxianus* CBS 6556	Baker's yeast[a]
1a[c]	**2a** (%)[b]	89	59	91	14	80	Quantitative	Quantitative
	Rt (h)	8	24	24	25	29	8	3
	De (%)[d]	81	83	56	32	32	>99	92
1b	**2b** (%)[b]	84	84	95	21	83	69	88
	Rt (days)	5	5	1	5 h	4	5	5
	De (%)[d]	None	None	40	n.d.	60	54	50
1c	**2c** (%)[b]	60	61	25	0	69	31	Quantitative
	Rt (days)	11	11	11	4	11	11	1.5
	De (%)[d]	60	66	>98	None	86	None	89
1d	**2d** (%)[b]	Quantitative	Quantitative	88	10	76	50	43
	Rt (days)	1	1	5	1	6	6	4
	De (%)[d]	94	91	>99	None	None	None	70
1f	**2f** (%)[b]	57	96	0	1	35	0	Quantitative
	Rt (days)	8	8	7	4	7	11	3
	De (%)[d]	>99	>99	None	54	None	None	>99
1g	**2g** (%)[b]	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	93	Quantitative
	Rt (days)	4	4	10 h	4	10 h	6	2
	De (%)[d]	4	3	4	6	3	52	2

[a] See Ref. 15.
[b] Percentage (%) of **2a–d** and **2f,g** formed (GC–MS quantitative analysis).
[c] See Refs. 15,16.
[d] *syn* > *anti* pair (^{1}H NMR data).

in the presence of baker's yeast. Diastereoisomeric excesses were very high, ranging from 91% to >99%, compared to the de = 70% for baker's yeast. No de was observed in the transformation of **1d** into **2d** performed in the presence of *Cr. curvatus*, *T. variabilis* and *K. marxianus*.

Diastereoisomeric excesses were still very high when **1f** was reduced in the presence of both strains of *S. cerevisiae* (de >99%). Although, after 8 reaction days, conversion decreased by using *S. cerevisiae* DSM 11285 (57%; quantitative conversion in 3 days for baker's yeast), it was again very high (96%), when *S. cerevisiae* CBS 7336 was employed.

The reduction of **1g** always proceeded with complete conversion into **2g**, as with baker's yeast. Reaction times were usually higher (4–6 days compared to 2 days of baker's yeast). Using *C. bombicola* and *T. variabilis*, reaction was even faster (10 h). The observed diastereoselectivity was comparable to that of baker's yeast (2%), with the exception of *K. marxianus*, which reduced **1g** to **2g** with a significantly higher de (52%).

It is noteworthy that in performing these bioreductions, other products **3b–d**, **3f**, **4b–d** and **4f**, (Scheme 2) formed, probably due to the simultaneous presence in the reaction medium of hydrolase(s) responsible for the hydrolysis of the ester function, followed by spontaneous decarboxylation of the intermediate 3-oxoacids,[17] affording ketones **3b–d** and **3f**, which were quickly bioreduced to **4b–d** and **4f** (Scheme 2). Compounds **3b–d**, **3f**, **4b–d** and **4f** did not form in blank experiments.[17]

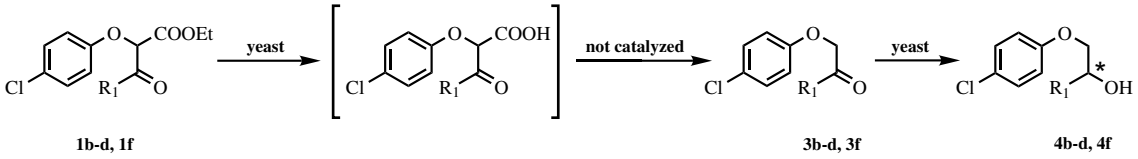
All microbes, with the exception of *Cr. curvatus*, showed after incubation times up to 5 days with **1b**, a moderate percentage of **3b** and **4b**, ranging between 5% (*C. bombicola*) and 26% (*K. marxianus*). *Cr. curvatus* rapidly converted almost all (79%) the substrate into **3b** and **4b** (Table 2).

The reduction of **1c** took longer reaction times (up to 11 days, Tables 1 and 2), this allowed the formation of significantly higher amounts of **3c** and **4c**, which ranged from 25% to 69%. Within 7 days, **3c** and **4c** were quantitatively formed in the presence of *Cr. curvatus*. No **3d** and **4d** formation was observed by reducing **1d** with both strains of *S. cerevisiae*. It is noteworthy that in all biotransformations, these two strains gave the lowest amounts of the secondary products (⩽32%).

With **1f**, very high amounts of **3f** and **4f** were obtained using *Cr. curvatus* (97%, within 4 days), *C. bombicola* (62%, within 7 days) and *T. variabilis* (60%, within 7 days), while with *K. marxianus*, 36% of these products were found.

On the other hand, compound **1g** showed a striking stability towards the enzymatic hydrolysis: only the expected product **2g** was always obtained. The presence of a methyl on C2 in **1g** probably does not allow a suitable interaction with the hydrolase active site.

In summary, among all the yeasts screened, *Cr. curvatus* expresses the highest level of hydrolytic enzyme(s) responsible for the formation of **3b–d** and **3f**. Bioreductions mediated by *T. variabilis* are not completely satisfactory even though product formation was acceptable with conversions ranging between 35% (**1f**) and quantitative (**1g**). Stereoselection was often low to fair (de = 3–60%), except for compound **1c**, for which the highest diastereoisomeric excess was observed (86%). Furthermore, similarly to *C. bombicola* and *Cr. curvatus*, *T. variabilis* displays remarkable hydrolytic activity towards all the reduced substrates. Both strains of *S. cerevisiae* had the same behaviour towards all of the compounds screened, showing a remarkable reducing activity and high conversions. Furthermore, baker's yeast still constitutes a good biocatalyst alternative, at least in the case of compounds **1b**, **1c** and **1f**. According



Scheme 2. Hydrolysis of **1b–d** and **1f**, decarboxylation of the produced acids to **3b–d** and **3f** reduced, then, to **4b–d** and **4f**.

Table 2. Reaction time (Rt) and percentage (%) of **3b–d**, **3f** and **4b–d**, **4f** formed (together with **2b–d**, **2f**) from **1b–d**, **1f** incubated in the presence of different yeasts

Yeast	**3b + 4b**		**3c + 4c**		**3d + 4d**		**3f + 4f**	
	Rt[a] (days)	%[b]	Rt[a] (days)	%[b]	Rt[a] (days)	%[b]	Rt[a] (days)	%[b]
Saccharomyces cerevisiae[c]	5	16	11	32	24	0	7	0
Cryptococcus curvatus	5 h	79	7	100	29	90	4	97
Candida bombicola	1	5	11	66	96	12	7	62
Trigonopsis variabilis	4	17	11	25	125	24	7	60
Kluyveromyces marxianus	5	26	7	69	101	45	7	36

[a] Reaction time (Rt) are those reported in Table 1.
[b] Percentage (%) of formation of **3** and **4** was determined in the reaction crude by GC–MS quantitative analysis chromatogram (see Section 4).
[c] Same results were obtained from both strains of *Saccharomyces cerevisiae*.

Table 3. Best yeast-mediated reduction conditions for **1a–d** and **1f,g** [reaction time (Rt), diastereoisomeric excess (De) values and percentage (%) of **2a–d** and **2f,g** formed]

Compd	Yeast	Rt (h)	Product	%	De (%)
1a	*Kluyveromyces marxianus*	8	**2a**	Quantitative	>99
1b	Baker's yeast	120	**2b**	88	50
1c	Baker's yeast	40	**2c**	Quantitative	89
1d	*Saccharomyces cerevisiae* DSM	24	**2d**	Quantitative	94
1f	Baker's yeast	72	**2f**	Quantitative	>99
1g	*Candida bombicola*	10	**2g**	Quantitative	4

to the reported data, the best yeast-mediated reduction conditions for each of the substrates **1b–d** and **1f,g** are reported in Table 3.

3. Conclusion

In conclusion, even though the synthesized substrates **1a–d** and **1f,g** have a very high degree of structural similarities, a specific yeast for the reduction of each of them was identified, enabling us to obtain **2a–d** and **2f,g** with high conversion (88% to quantitative) and low to very good diastereoselectivities (4% to >99%). This justifies the continuous effort aimed at finding new microorganisms for the preparation of valuable enantiomerically pure compounds for fine chemicals and pharmaceuticals.

4. Experimental

Reaction progress was monitored by TLC and GC analysis. Thin-layer chromatography (TLC) was performed on silica gel sheets with a fluorescent indicator (Statocrom SIF, 60 F_{254} Merck); TLC spots were observed under ultraviolet light or visualized with I_2 vapour. Column chromatography was performed using silica gel Merck 60 (0.063–0.200 μm). GC analyses were performed by using a HP5 column (methyl silicone gum; 30 m × 0.25 mm × 250 μm film thickness) on a Hewlett Packard 5890 model, Series II. GC–MS analyses were performed on a Hewlett Packard 6890-5793MSD.

Compounds **1b–g** were prepared as reported.[16] Spectroscopic data for **2b–g**,[18] **3b–d**, **3f** and **4b–d** and **4f**[19–23] are consistent with those ones reported. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Mercury 300 MHz spectrometer.

Optical density was measured at 620 nm by a Perkin–Elmer UV/Vis Lambda Bio 20 spectrophotometer.

4.1. Microorganism sources

The baker's yeast used to reduce 3-oxoesters **1a–g** was supplied by Lievitalia. *K. marxianus* CBS 6556, *S. cerevisiae* DSM 11285, *S. cerevisiae* CBS 7336, *Cr. curvatus* ATCC 20509, *C. bombicola* ATCC 22214 and *T. variabilis* DSM 70714 were obtained from public type culture collections (ATCC, CBS, DSM).

4.2. Culture medium

The microorganisms were cultivated under aerobic conditions in a medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose. Agar-agar (2%) was added to the same medium for cells preservation on agar slants.

4.2.1. Bioreduction of 1b–f using yeasts: general procedure. Cells preserved on agar slants at 4 °C were used to inoculate 250 mL flasks containing 100 mL of the culture medium. The flasks were incubated aerobically at 30 °C on an orbital shaker and stirred at 250 rpm. Flasks (250 mL) containing 100 mL of the culture medium were then inoculated with 5 mL of the 24-h-old suspension and incubated in the same conditions for 24 h. Flasks (1 L) containing 400 mL of the culture medium were then inoculated with 5 mL of the latter suspension and incubated for 24 h. Ethyl 3-oxoesters **1b–g** (200 mg) dissolved in 1 mL of ethanol were added after all cultures have the optical density reported in Table 4. Reaction was followed by GC analysis and stopped at the times indicated in Tables 1 and 2. The content of the flask was then centrifuged and the supernatant extracted with ethyl acetate. All the reactions were repeated at least twice without any noticeable bias in the results.

Table 4. Culture medium optical density at which **1b–g** have been added

Yeast	Optical density[a]
Saccharomyces cerevisiae CBS 7336	4.9
Saccharomyces cerevisiae DSM 11285	4.5
Trigonopsis variabilis DSM 70714	4.4
Cryptococcus curvatus ATCC 20509	8.2
Candida bombicola ATCC 22214	8.8
Kluyveromyces marxianus CBS 6556	7.2

[a] Measured at 620 nm.

4.2.2. Blank experiment. A 1 L flask containing 400 mL of the culture medium was stirred at 30 °C on an orbital shaker at 250 rpm. Ethyl 3-oxoesters (200 mg) **1b–f** dissolved in 1 mL of ethanol were added. The reaction was monitored by GC analysis and stopped after 13 days. The content of the flask was extracted with ethyl acetate and analyzed.

4.2.3. Analytics. The reaction mixture (0.5 mL) was extracted by adding ethyl acetate (1 mL). The sample was centrifuged at 10,000*g* for 5 min in order to separate the

two phases. The supernatant was dried by anhydrous Na_2SO_4 and then analyzed by GC and/or TLC.

Acknowledgements

The work was carried out under the framework of the National Project 'Progettazione, Sintesi e Valutazione Biologica di Nuovi Farmaci Cardiovascolari' supported by the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR, Rome). Thanks are also due to the University of Bari and CNR-Istituto di Chimica dei Composti OrganoMetallici-ICCOM/Sezione di Bari (Italy).

References

1. Sgro, C.; Escousse, A. *Therapie* **1991**, *46*, 351–354.
2. Gariot, P.; Barrat, E.; Mejean, L.; Pointel, J.; Drouin, P.; Debry, G. *Arch. Toxicol.* **1983**, *53*, 151–163.
3. Angelin, B.; Einarsson, K.; Leijd, B. *Eur. J. Clin. Invest.* **1984**, *14*, 73–78.
4. Rangwala, S. M.; O'Brien, M. L.; Tortorella, V.; Longo, A.; Loiodice, F.; Noonan, D. J.; Feller, D. R. *Chirality* **1997**, *9*, 37–47.
5. Ferorelli, S.; Loiodice, F.; Tortorella, V.; Conte-Camerino, D.; De Luca, A. *Farmaco* **2001**, *56*, 239–246.
6. Carbonara, G.; Fracchiolla, G.; Loiodice, F.; Tortorella, P.; Conte-Camerino, D.; De Luca, A.; Liantonio, A. *Farmaco* **2001**, *56*, 749–754.
7. Liantonio, A.; Accardi, A.; Carbonara, G.; Fracchiolla, G.; Loiodice, F.; Tortorella, P.; Traverso, S.; Guida, P.; Pierno, S.; De Luca, A.; Conte-Camerino, D.; Push, M. *Mol. Pharmacol.* **2002**, *62*, 265–272.
8. Feller, D. R.; Kamanna, V. S.; Newman, H. A. I.; Romstedt, K. J.; Witiak, D. T.; Bettoni, G.; Bryant, S. H.; Conte-Camerino, D.; Loiodice, F.; Tortorella, V. *J. Med. Chem.* **1987**, *30*, 1265–1267.
9. Bettoni, G.; Loiodice, F.; Tortorella, V.; Conte-Camerino, D.; Mambrini, M.; Ferrannini, E.; Bryant, S. H. *J. Med. Chem.* **1987**, *30*, 1267–1270.
10. Esbenshade, T. A.; Kamanna, V. S.; Newman, H. A. I.; Tortorella, V.; Witiak, D. T.; Feller, D. R. *Biochem. Pharmacol.* **1990**, *40*, 1263–1274.
11. Inomata, N.; Yoshida, H.; Aoki, Y.; Tsunoda, M.; Yamamoto, M. *Tohoku J. Exp. Med.* **1991**, *165*, 171–182.
12. Ciolek, E.; Dauca, M. *Biol. Cell.* **1991**, *71*, 313–320.
13. Ferorelli, S.; Franchini, C.; Loiodice, F.; Perrone, M. G.; Scilimati, A.; Sinicropi, M. S.; Tortorella, P. *Tetrahedron: Asymmetry* **2001**, *12*, 853–862, and references cited therein.
14. Perrone, M. G.; Santandrea, E.; Di Nunno, L.; Scilimati, A.; Tortorella, V.; Capitelli, F.; Bertolasi, V. *Tetrahedron: Asymmetry*, **2005**, *16*, 783–792.
15. Perrone, M. G.; Santandrea, E.; Scilimati, A.; Tortorella, V.; Capitelli, F.; Bertolasi, V. *Tetrahedron: Asymmetry* **2004**, *15*, 3501–3510.
16. Perrone, M. G.; Santandrea, E.; Scilimati, A.; Syldatk, C.; Tortorella, V.; Capitelli, F.; Bertolasi, V. *Tetrahedron: Asymmetry* **2004**, *15*, 3511–3517.
17. Hydrolysis of 3-oxoesters **1a–d** and **1f** by LiOH/THF–H_2O affords 3-oxoacids, which quickly and spontaneously undergo decarboxylation (as many carboxylic acids bearing at the α-position a strong electron withdrawing group behave) to the corresponding ketones **3a–d** and **3f**. Conversely, the hydrolysis of the 3-hydroxyesters **2a–d** and **2f** into the corresponding 3-hydroxyacids by KOH/THF–H_2O was not followed by their decarboxylation, and it was possible to isolate the stable 3-hydroxyacids.
18. Perrone, M. G.; Santandrea, E.; Dell'Uomo, N.; Giannessi, F.; Milazzo, F. M.; Sciarroni, A. F.; Scilimati, A.; Tortorella, V. *Eur. J. Med. Chem.* **2005**, *40*(2), 143–154.
19. Eberle, M.; Stierli, D.; Mueller, U. PCT WO 2004000797, *Chem. Abstr.* 140:59414.
20. Ogawa, K.; Terada, T.; Muranaka, Y.; Hamakawa, T.; Hashimoto, S.; Fujii, S. *Chem. Pharm. Bull.* **1986**, *34*, 1118–1127.
21. Imai, T.; Takao, H.; Yamaguchi, K.; Uchida, T.; Goto, T.; Umetsu, N. *Pestic. Sci.* **1992**, *34*, 119–125.
22. Luerssen, K.; Brandes, W.; Haenssler, G.; Holmwood, G.; Reinecke, P. DE3325761, 1985.
23. Boeckmann, K.; Regel, E.; Buechel, K. H.; Luerssen, K.; Konze, J.; Brandes, W. CA1257263, 1989.