Synthesis and Biological Activity of Derivatives of the Herbicidal Metabolite CL22T (Phthoxazolin)

FRÉDÉRIC LEGENDRE[†], MARIE DOLORÈS MATURANO[††], GILLES ETIENNE[†††], ALAIN KLAEBE[††] and GÉRARD TIRABY[†††,*]

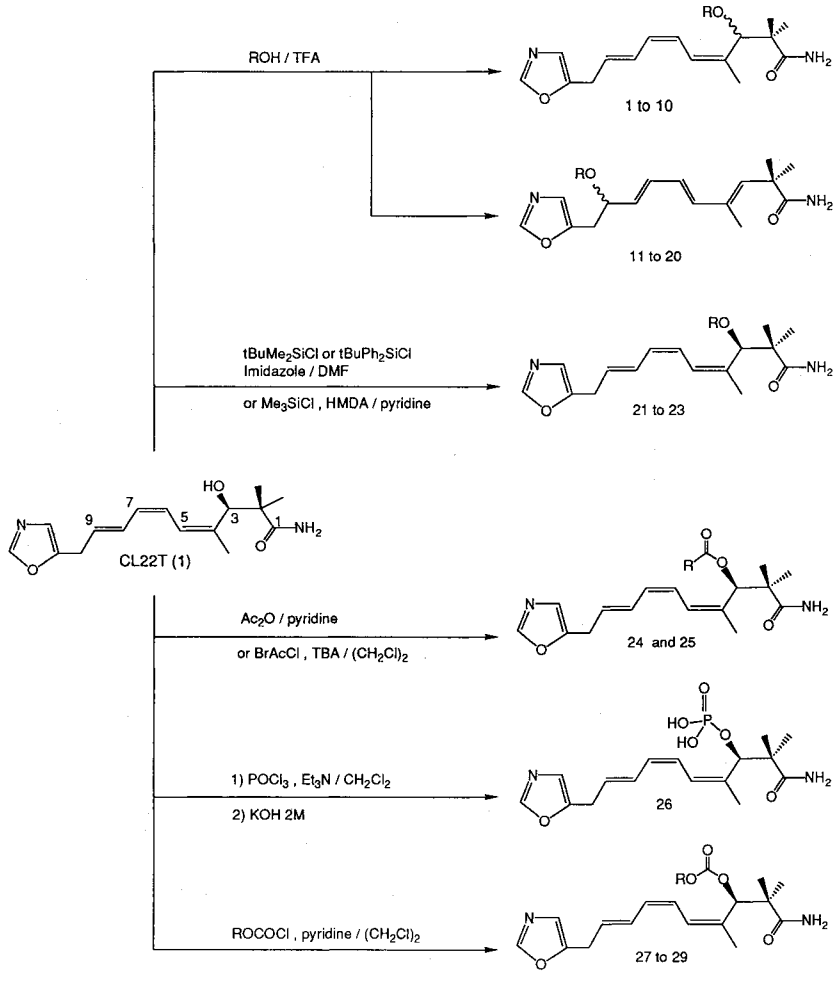
[†]Laboratoires CAYLA, Avenue de Larrieu, 31094 Toulouse cedex, France
[††]Laboratoire S.S.R.M.P., Groupe de Chimie Organique Biologique, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex, France
[†††]Laboratoire de Microbiologie et Génétique appliquées, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex, France

(Received for publication June 9, 1994)

During a screening program for Actinomycetes strains producing pesticidal substances with weak or no antimicrobial activity, a herbicidal product, CL22T (**1**) was isolated from the fermentation broth of *Streptomyces griseoaurantiacus*[1,2]. It was originally screened as an inhibitor of germination and as a contact herbicide specific for dicotyledon plants. In 1990, ŌMURA *et al.*[3] described phthoxazolin, a phytotoxic compound identical to CL22T. To assess the potential of CL22T (**1**) to provide a new family of herbicides, we investigated structure-activity relationships by introduction of substituents or modifications at possible sites of the molecule, such as the hydroxy group (Scheme 1), the primary amide function or the oxazole ring (Scheme 2). Derivatives were purified by HPLC on reverse phase for the non-ionic compounds, or by ion exchange on Sep Pack cartridges for the ionic ones and their purity checked by HPLC and TLC using at least two different mobil phases. Structural data were obtained by UV, ^{1}H NMR or EI mass spectrometry and biological activity was assayed on the growth of radish seedlings.

The allylic hydroxy group was alkylated using various alcohols and TFA in acetonitrile as described by TANAKA *et al.*[4] (compounds **2** to **20**). This alkylation method generated two different compounds[5] in each reaction leading to the alkylated hydroxy derivatives (**2** to **10**) and to alternative products arising from the allylic alcohol transpostion (**11** to **20**). The EI mass spectrum of CL22T (**1**) showed the formation of a major fragment ion at *m/z* 204 due to the *β*-ketol cleavage between C-2 and C-3. For the alkylated derivatives **2** to **10**, the same

Scheme 1. Derivatives of the allylic hydroxy group of CL22T.



Scheme 2. Amide and substituted oxazole ring derivatives of CL22T.

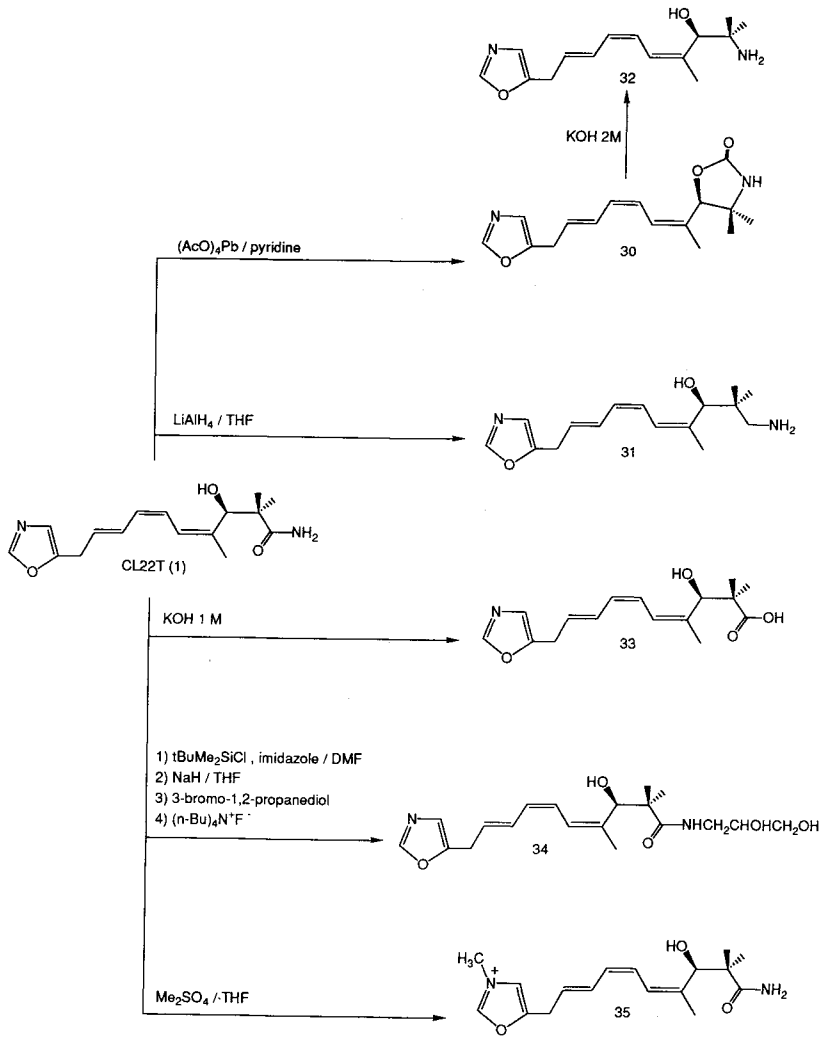


Table 1. Nature of R substituent in Schemes 1 or 2.

Product	R=
1 or **11**	H
2 or **12**	CH_3
3 or **13**	CH_2-CH_3
4 or **14**	$CH_2-CH_2-CH_3$
5 or **15**	$CH-(CH_3)-CH_3$
6 or **16**	$CH_2-(CH_2)_2-CH_3$
7 or **17**	$CH-(CH_3)-CH_2-CH_3$
8 or **18**	C_6H_5
9 or **19**	$CH_2-(CH_2)_6-CH_3$
10 or **20**	CH_2-CH_2SH
21	$Si(CH_3)_3$
22	$Si(CH_3)_2-C(CH_3)_3$
23	$Si(C_6H_5)_2-C(CH_3)_3$
24	CH_3
25	CH_2-Br
27	CH_3
28	CH_2-CH_3
29	$C_6H_4-NO_2$

type of fragmentation occurred and led to a fragment ion increased by the number of mass units corresponding to the introduced alkyl group. The EI fragmentation of **11** obtained by allylic transposition of **1** displayed a main fragment ion at *m/z* 208 due to the cleavage between C-9 and C-10. This ion fragment was also dominant for the alkylated compounds **12** to **20**. Silyl ether derivatives (**21** to **23**) were synthesized with trimethylchlorosilane and hexamethyldisilazane in pyridine, or with *tert*-butyldimethylchlorosilane or *tert*-butyldiphenylchlorosilane in DMF in the presence of imidazole. Acylated derivatives **24** and **25** were obtained respectively by acetylation of CL22T (**1**) with acetic anhydride in pyridine or by acylation with bromoacetylchloride in dichloroethane in the presence of tribenzylamine. Phosphorylation of the allylic hydroxy group was carried out using phosphorous oxychloride in dichloromethane in presence of triethylamine followed by basic hydrolysis yielding **26**. Carbonated derivatives **27** to **29** were obtained by reaction of methyl, ethyl or *p*-nitrophenyl chloroformates respectively in dichloroethane and pyridine. The nature of the R substituents of compounds **1** to **29** described above is indicated in Table 1.

CL22T (**1**) was also oxidized with lead tetraacetate in pyridine to provide **30** resulting from a Hofmann-like

Table 2. Activity of CL22T derivatives against growth of radish seedlings.

Product	IC 20[a]	Product	IC 20[a]
1	0.5	**19**	>125
2	>125	**20**	>125
3	>125	**21**	2
4	>125	**22**	>125
5	>125	**23**	>125
6	>125	**24**	20
7	>125	**25**	>125
8	>125	**26**	>125
9	>125	**27**	>125
10	>125	**28**	>125
11	>125	**29**	100
12	>125	**30**	5
13	>125	**31**	50
14	>125	**32**	100
15	>125	**33**	100
16	>125	**34**	>125
17	>125	**35**	>125
18	>125		

[a] Minimal concentration to inhibit 20% of the growth of radish seedlings.

rearrangement between the primary amide and β-allylic alcohol functions as described by SIMONS[6]. The oxazolidinone ring of **30** was hydrolyzed in the presence of potassium hydroxide to the amine **32**. The primary amide function was reduced with lithium aluminium hydride in THF giving the amine **31**. The hydrolysis of **1** in basic conditions provided the free acid **33**. Product **22** was also treated with sodium hydride in THF and alkylated with 3-bromo-1,2-propanediol. The hydroxy function was deprotected by treatment with tetra-*n*-butylammonium fluoride to yield **34**. The nitrogen of the oxazole ring of CL22T (**1**) was methylated with dimethylsulfate in THF resulting in **35**.

The herbicidal activity of all derivatives was assayed on radish seeds growing in 24-well plates. A paper disk was placed in each well moistened with 200 μl of solution containing the test compound. Each compound was tested at different concentrations in 4 wells with 3 seeds per well incubated at 22°C. The seedlings were moistened with 200 μl per well every 2 days and the lowest concentration required to inhibit the growth of seedlings was determined after one week. From the results summarized in Table 2, it clearly appears that the allylic alcohol group was required for herbicidal activity because most of the chemical modifications on this function entailed a complete loss of phytotoxicity. In addition, all the products resulting from the allylic transposition were inactive. All hydroxy derivatives that preserved some phytotoxicity are products that can chemically or biologically regenerate the native CL22T (**1**). The trimethylsilyl ether bond of **21** was unstable in aqueous solution, as is the case for **29** for which the carbonate bound is weakened by the *para* nitro group at the phenyl ring. Compound **24** with the acetylated hydroxyl retained a weak activity against seedlings probably due to the presence of esterases. When the primary amide group was reduced to the primary amine (**31**~**32**) or hydrolyzed as acid (**33**), the derivatives preserved some but very weak phytotoxicity. Remarkably, compound **30**, characterized by an oxazolidinone ring, showed a significative inhibitory activity against seedlings compared to the other derivatives of CL22T (**1**) although the allylic alcohol and primary amide functions were both modified. This can be explained by the similarity between the oxazolidinone ring and the geometry of the terminal moiety of CL22T (**1**). IR and NMR studies and molecular mechanics models using the MACROMODEL V 3.0. program (Prof. W. C. STILL, Columbia University, New York, U.S.A.) demonstrated the existence of a strong hydrogen bond between the oxygen of the alcohol function and one hydrogen of the primary amide group in **1** resulting in a quasi ring structure.

The total synthesis of CL22T is underway with a different approach to that recently described for the synthesis of neooxazolomycin[7], a phthoxazolin condensation compound, to provide a new series of more highly modified compounds hopefully with phytochemical activity.

Acknowledgements

We thank the Ministère de la Recherche et de la Technologie for financial support (Grant 88-T-0849) and F. DROCOURT, CAYLA Toulouse, for help in typing this manuscript.

References

1) FABRE, B.; E. ARMAU, G. ETIENNE, F. LEGENDRE & G. TIRABY: A simple screening method for insecticidal substances from Actinomycetes. J. Antibiotics 41: 212~219, 1988
2) LEGENDRE, F. & E. ARMAU (CAYLA SARL): Nouveaux composés herbicides, le CL22T et ses dérivés, procédés de préparation de ces composés, composition les contenant et procédés de traitement du desherbage les utilisant. French Patent 89,08615, June 28, 1989
3) ŌMURA, S.; Y. TANAKA, I. KANAYA, M. SHINOSE & Y. TAKAHASHI: Phthoxazolin, a specific inhibitor of cellulose biosynthesis, produced by a strain of *Streptomyces* sp. J. Antibiotics 43: 1034~1036, 1990
4) TANAKA, A.; A. WATANABE, R. KOBAYASHI, T. TSUCHIYA, S. UMEZAWA, M. HAMADA & H. UMEZAWA: Synthesis of 9-substituted josamycin, 13-substituted isojosamycin and their tetrahydro derivatives. J. Antibiotics 34: 1137~1151, 1981
5) OMURA, S.; M. KATAGIRI, H. OGURA & T. HATA: The chemistry of leucomycins. III. Structure and stereochemistry of leucomycin A_3. Chem. Pharm. Bull. 16: 1181~1186, 1968
6) SIMONS, S. S.: Lead tetraacetate and pyridine. New mild conditions for Hofmann-like rearrangement. A new synthesis of 2-oxazolidinones. J. Org. Chem. 38: 414~416, 1973
7) KENDE, A. S.; K. KAWAMURA & R. J. DEVITA: Enantioselective total synthesis of neooxazolomycin. J. Am. Chem. Soc. 112: 4070~4072, 1990