Pure & Appl. Chem., Vol. 65, No. 6, pp. 1233–1240, 1993.
Printed in Great Britain.

Structural elucidation and chiral syntheses of insect pheromones and extension of the synthetic approaches

Lin, Guo-qiang

Shanghai Institute of Organic Chemistry
Chinese Academy of Sciences, Shanghai 200032, China ; FAX: 021-4335712

Abstract: Structural elucidation and chiral synthesis of insect pheromone are described. The synthetic work was extended to the ring opening of an epoxide with amines and mercaptans, and to the microbial reduction of α or β-diketonic compounds by *Geotrichum* sp.38.

Insect pheromone, a mixture of chemicals released from one organism and inducing a response by another individual of the same species, is another interesting world in the field of natural products. Pheromones are characterized by numerous variations with very high bioactivity, and by the critical chemo- and chiral recognition exhibited by insects. With the increasing number of identified chiral pheromones, more attention has been paid to the topic of stereochemistry--activity relationship. Therefore, there is a demand for stereoselective and enantiospecific syntheses of insect pheromones and this leads to one of the general subjects in synthetic organic chemistry.

Due to the insufficient supply of natural pheromones, some structural determination techniques must be adapted to the 10 ng level. Six economically important insect species were targeted for pheromone identification, and studied by means of ethereal washing of the insect pheromone gland of female moths, GC-MS detection, electroantennogram (EAG) survey and single cell recording (SCR).

For synthesizing optically active pheromones and their stereoisomers, usually three kinds of methods, i.e. chiron approach, asymmetric synthesis and microbial transformation were employed. We wish to illustrate these methods by examples.

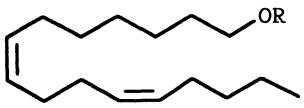
1. STRUCTURAL DETERMINATION

a. Pink bollworm moth, *Pectinophora gossypiella* (ref. 1)

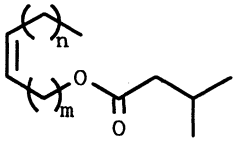
Determination of the isomeric ratio of the sex pheromone was performed by GC-MS and the result was verified through field tests. It revealed that the virgin female moths produce pheromones 1a and 1b in ratio of 58-55 to 42-45, and the other two geometric isomers (7E,11E and 7E,11Z) were not detected. Interestingly, the mated females produce free alcohols 2a and 2b in the same proportions as the acetates, and these alcohols were proved to be the natural pheromone inhibitors (ref. 2).

When the synthetic pheromone was incubated with the homogenized female pheromone glands, 2a+2b were obtained, indicating that there is an enzyme in the gland which converts the pheromone to its inhibitor. The same result was also observed when the male homogenized smashed antenna

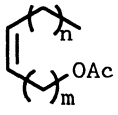
was incubated with the synthetic pheromone. Thus 2a and 2b can be considered as the metabolites of the pheromone (ref. 3).



1a 7Z,11Z, R = Ac
b 7Z,11E, R = Ac
2a 7Z,11Z, R = H
b 7Z,11E, R = H



3a 7Z, m=6, n=9
b 7Z, m=5, n=10
c 7Z, m=5, n=10, n-valerate
d 7Z or 7E, m=8, n=7



4a 9E, m=8, n=1
b 9Z, m=8, n=1
5a 7E, m=6, n=3
b 7Z, m=6, n=3

6a 3E,13Z, R = H
b 3Z,13Z, R = H
c 3Z,13E, R = H
d 3E,13E, R = H
e 3E,13Z, R = Ac
f 3Z,13Z, R = Ac

b. *Euproctis similis xanthocampa*

Four components, i.e. Z-7-octadecenyl isovalerate (92%, 3a), 6-octadecenyl isovalerate (1.4%, 3b), 6-octadecenyl n-valerate (1.6%, 3c) 9-octadecenyl isovalerate (5%, 3d) were identified in the sex gland of this insect, a major pest of the mulberry tree. Only the synthetic 3a was found to be as active as the female moth in the field bioassay. All other isomers including the E isomer of 3a, and the normal valerates of 3b and 3d were inactive. 3a is a new structure, and the isovalerate type used as pheromone was found in Lymantridae for the first time (ref. 4).

c. *Ancylis sativa* Liu

Four structurally related compounds 4a-b, 5a-b were identified from the sex pheromone gland of the female moths, a leafroller of the Chinese date. The pheromone amounted to 2.4 μg per female moth. The mixture of 4a and 4b demonstrated a strong attraction in the field tests only when the ratio of 4a/4b was 8/2, whereas 5a and 5b were inactive and did not exhibit any inhibitory or synergic effect (ref. 5). The establishment of the 4a-b pheromone structure is an alternative evidence, from the taxonomic point of view that the name *Ancylic sativa* which was taxonomically classified by Liu, is correct, the previous one *Cerostoma sasaki* by Matsumura being erroneous (ref. 6).

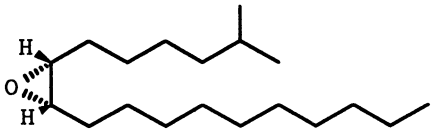
d. *Paranthrane rabaniformis* R. and Sesiidae

The pheromones from six species-relevant clearwing moth (sesiidae), a serious forest pest were examined. 6a was identified as the sex pheromone of poplar twig clearwing moth *P. rabaniformis* R. (ref. 7). A similar species, poplar large hornet moth, *Sphecia siningensis* H. uses 6b as its sex signal (ref. 8). From the pheromone gland of the female mulberry tree borer *Paradoxes prelle* L., the acetate 6e and the corresponding alcohol 6a, averaging 250 ng and 30 ng per female moth, were identified, and only 6e was an attractant in the field tests (ref. 9). SCR investigation showed clearly

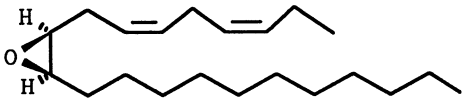
that each male *sensilla trichodeum* containe two kinds of receptor, one responding to 6e and producing large spikes; the other one is sensitive to 6f and produced smaller spikes. 6a is also a major pheromone component of the vine tree borer *P. regalis* B. (ref. 10) and an attractant to *S. castanerora* and *C. hector*.

From the cases of the sesiidae together with those of the above three species, one can easily draw the conclusion that the pheromone system is really complex. Although five species of clearwing moth share 6a as pheromone or at least as attractant, there must be, in order to keep species isolation, other type of cells in the antenna of each species which respond to other minor components. SCR investigation showed there are two types of recepors of *P. rabaniformis* receiving 6a and 6b respectively; in the case of *P. regalis* , two kind cells corresponded to 6a and 6b making large spikes, other two kinds receptor made small spikes induced by 6d and 6e, respectively.

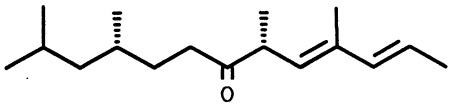
2. CHIRAL SYNTHESIS



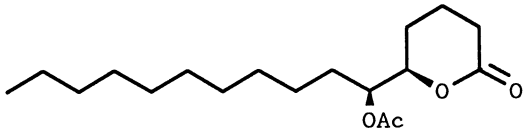
7 7R, 8S



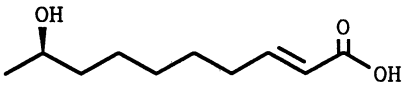
8 9S, 10R



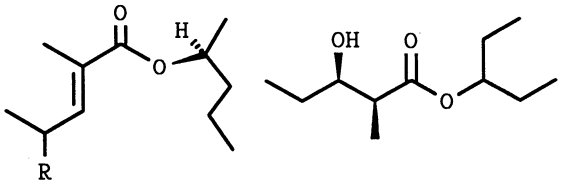
9 6R, 10R



10 5R, 6S



11 9R



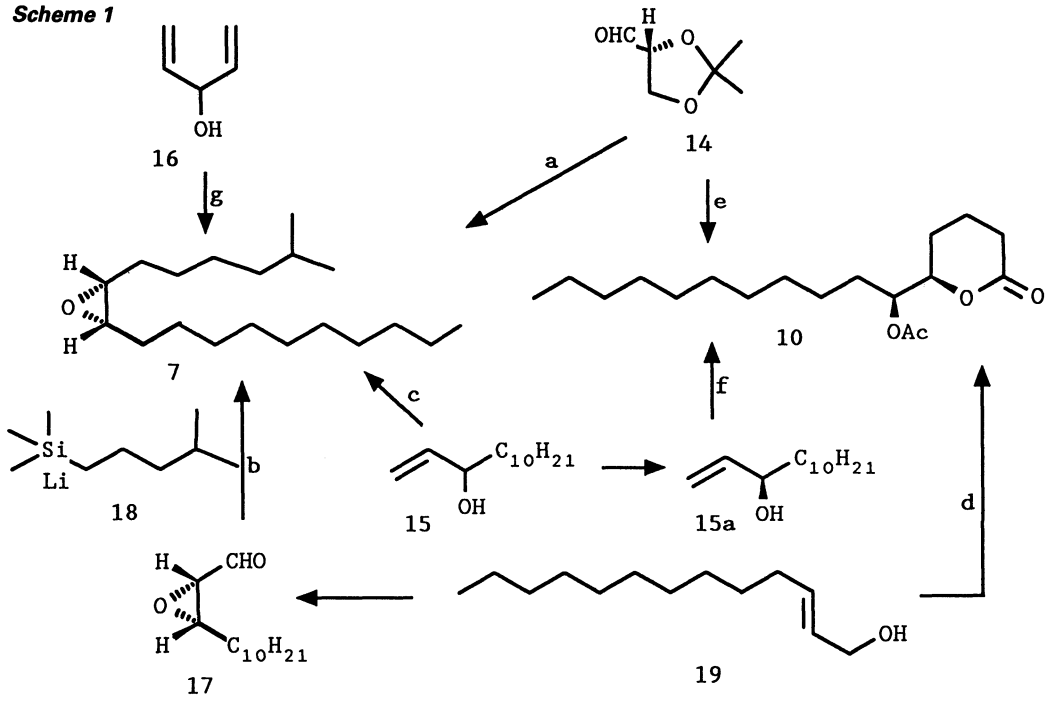
12 2S a, R=H
b, R=Me

13 2S, 3R

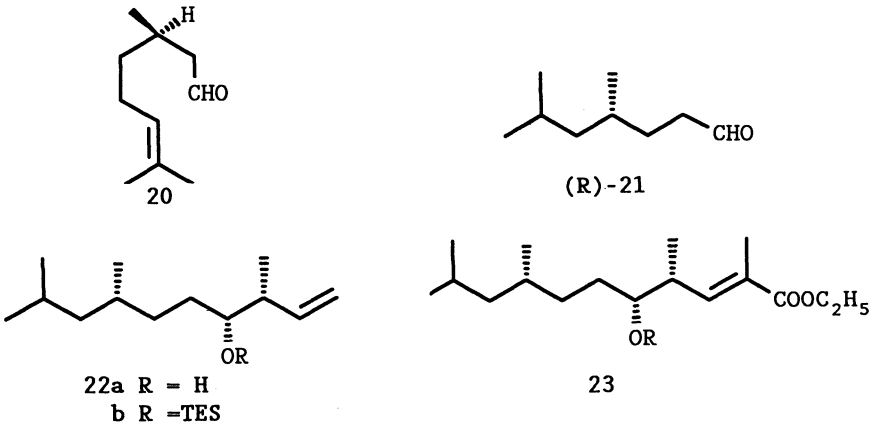
We selected 7-13 as our target molecules as they were pheromone of some important pests. 7, 8 and 9 were identified as the sex pheromones of three species of forest insects, i.e. the gypsy moth *Porthetria dispar* (ref. 11), the american white moth *Hyphantrie cunea* D. (ref. 12), and the pine tree scale *Matsucoccus matsumurae* (ref. 13), respectively. The absolute configuration of 9 was so far not settled yet (ref.13). 10 is the mosquito oviposition attractant pheromone for *Culex pipiens fatigens*, identified by Laurence and his co-workers in 1982 (ref. 14). 11 is the queen substance of the Italian honey bee *Apis mellifera* L. The R form of 11 is more active than its S form (ref.15). 12 and 13 are aggregation pheromones for the lesser grain borer *Phyzopotha dominica* (ref. 16) and grainary weevil *Sitophilus granarius* (ref. 17), respectively. Both 12a and 12b are active (ref. 16).

a. Chiron approach and asymmetric synthesis

Scheme 1



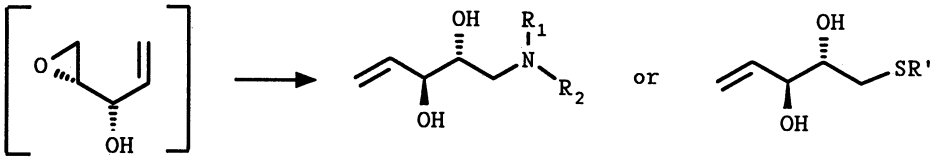
2,3-O-isopropylidene(+)-R-glyceraldehyde 14, prepared according to ref. 18, was used to synthesize all four stereoisomers of 7 (ref. 19) (Scheme 1, a). Only (+)-7R,8S-7 was active in the field bioassay, the other isomers were inactive (ref. 20), indicating that male gypsy moths showed chiral recognition to 7. 7 was also obtained from 17 and 15 via the Sharpless Epoxidation Reaction (ref. 12) and its kinetic resolution (ref.22) (Scheme 1, b, c), respectively. All four stereoisomers of 6-acetoxy-5-hexadecanolide 10, the mosquito oviposition attractant pheromone were synthesized via the Sharpless Asymmetric Epoxidation (ref. 23) (Scheme 2, d). Only (-)-(5R, 6S)-10 was active in attracting *Culex pipiens fatigens* females for oviposition at dosage of 0.5 μg/100 ml H_2O. (5R,6S)-10 is 1/100 times less ovipositionally attractive to *C.tarsalis* and inactive to *Aedes aegypti* L. and *Anopheles quadrimacutates*, indicating genus specificity of the pheromone (ref. 24). 10 was also prepared from 14 (ref. 25) and 15a (ref. 26), 15a is the other product remaining after kinetic resolution of 15. (6R, 10R)-9 was obtained via 22 and 23 (ref. 27a). As Roush's Aldol Reaction



(ref. 27b) was used as the key step (21→22) to form the C-6 chirality of 9 and (R)-21 and (S)-21 forms could be prepared starting from (R)--citronellal 20, therefore both (6R, 10R)-9 and its enantiomer could be prepared.

Field tests showed that (6R, 10R)-9 is active; the preparation of (6S, 10S)-9 is in progress. As an extension to our discovery that addition of catalytic amounts of CaH_2 and siliga gel to the Sharpless reagent reduced the reaction time(ref.28), the divinylcarbinal 16 (ref.29) was subjected to the Sharpless Epoxidation and followed by *in situ* silylation to produce finally (7R, 8S)-7 (ref. 30) and (9S, 10R)-8 (ref. 31) with >99% e.e. purity.

Scheme 2



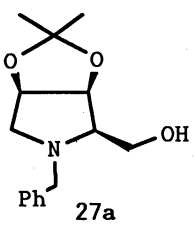
24 25 26

Table 1

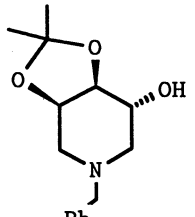
Entry	Nu	R_1	R_2	Entry	Nu	R_1
a	$HN(C_2H_5)_2$	C_2H_5	C_2H_5	h	PHSH	Ph
b	$HN(i\text{-}C_3H_7)_2$	$i\text{-}C_3H_7$	$i\text{-}C_3H_7$	i	$PhCH_2SH$	$PhCH_2$
c	H_2NCH_2Ph	PhCO	CH_2Ph	j	$2\text{-}NH_2PhSH$	NH_2Ph
d	$n\text{-}C_4H_9NH_2$	PhCO	$n\text{-}C_4H_9$	k	2-OHPhSH	2-OHPh
e	$PhCH_2ONH_2$	H	OCH_2Ph	l	$HS(CH_2)_2SH$	$HS(CH_2)$
f	$2,4\text{-}(CH_3)_2PhNH_2$	H	$2,4\text{-}(CH_3)_2Ph$	m	PHCOSH	PhCO
g	$3,4\text{-}(CH_3)_2PhNH_2$	H	$3,4\text{-}(CH_3)_2Ph$			

As an extension to the study of the properties of epoxides such as 24, the *in situ* nucleophilic ring opening of 24 with various amines and mercaptans was investigated (Scheme 2). The results are summarized in Table 1 (ref. 32). In the case of primary alkyl amine (Table 1, c and d), the corresponding products 25c, 25d were not stable. Thus, the *in situ* N-acylation right after amionlysis was performed. Substituted anilines were good nucleophiles (Table 1, f, g): 24g could be prepared without protection in 63% yield, while only about 26% yield was obtained when 24 was isolated (16→ 24, 70% yield; 24→ 25, 40% yield) (ref. 33).

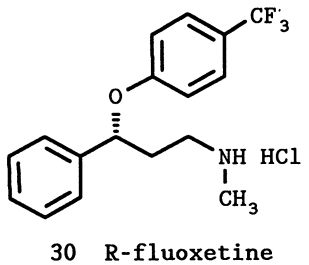
25c was a good building block which could be expected to undergo intermolecular electrophilic addition to form polyhydroxypyrrolidines or piperidine, such as 27a, 27b (ref. 34).



27a



27b

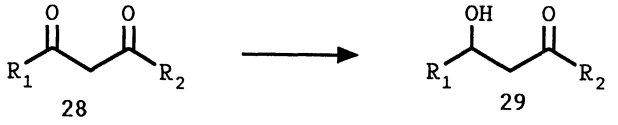


30 R-fluoxetine

b. Microbial transformation

In the course of broad screening of a variety of microorganisms for their ability to perform reduction reactions with high yields and opposite enantioselectivity in comparison with baker's yeast, we found *Geotrichum* sp. 38 (G.38), a fungus isolated from soil samples, a valuable biocatalyst in this regard. Table 2 (ref. 35) is an example, where reduction of 28 with G 38 yielded (R)-29, whereas baker's yeast produced (S)-form of 29.

Table 2



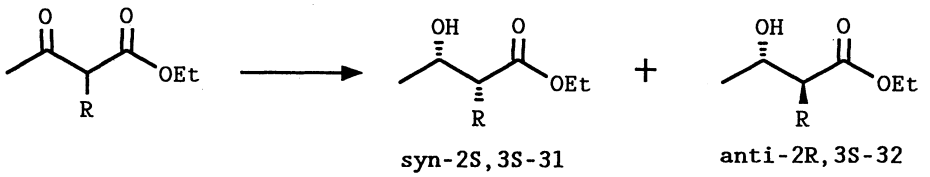
Entry	R_1	R_2	with baker's yeast				with G38			
			time(h)	yield%	e.e	Conf.	time(h)	yield%	e.e	Conf.
a	Me	Me	36	16	82	S	12	21	92	R
b	Et	Me	48	23	88	S	15	38	95	R
c	Pr	Me	48	32	75	S	18	53	99	R
d	Bn	Me	48	42	91	S	20	77	99	R
e	Pen	Me	60	46	86	S	24	75	47	R
f	Ph	Me	48	70	95	S	24	94	95	R
g*	Ph	OEt	48	86	99	S	48	91	81	R

* immobilized G38 and resting cell of baker's yeast were employed.

From 29 g, both (R)- and (S)-Fluoxetine 30 were prepared. Fluoxetine, the serotonin-uptake inhibitor, is one of the most exciting new therapeutic agent. (ref. 36) (see Fig. 1).

An enzyme — NADP (H) — requiring alcohol dehydrogenase — was isolated and purified from G38, which showed potential utility. When α-substituted ethyl acetoacetate. (Table 3) was reduced with this fresh-prepared enzyme, Aldol products were obtained with absolute syn-selectivity (ref. 35).

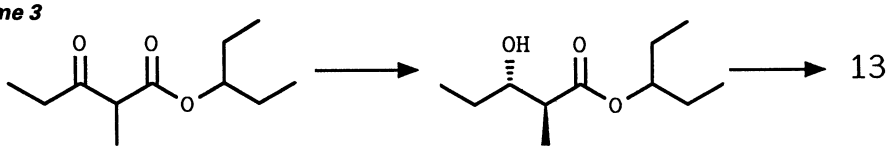
Table 3



Entry	R	time (day)	syn : anti
a	Me	2	100 : 0
b	Et	3	100 : 0
c	All	1	100 : 0
d	Bu	3	100 : 0
e	Pen	3	100 : 0

However, the suitable reaction condition range of this enzyme was not large enough (<30°C, PH=8). Thus, for convenient and routine work the whole cell of G38 was used. For example, the preparation of (3S,4R)-13,aggregation pheromone of a grainary weevil *Sitophilus granarius* (ref. 17) is shown in scheme 3.

Scheme 3



Reduction of 33 with G38 yielded 56% anti (2S, 3S)-34 (anti : syn= 97.4 : 2.6) which could be easily transformed to 13. The biotransformation using G38 to prepare (3S,4R)-13, 9R-11, 9S-11 and some useful drugs was summarized in Fig. 1 (ref. 35).

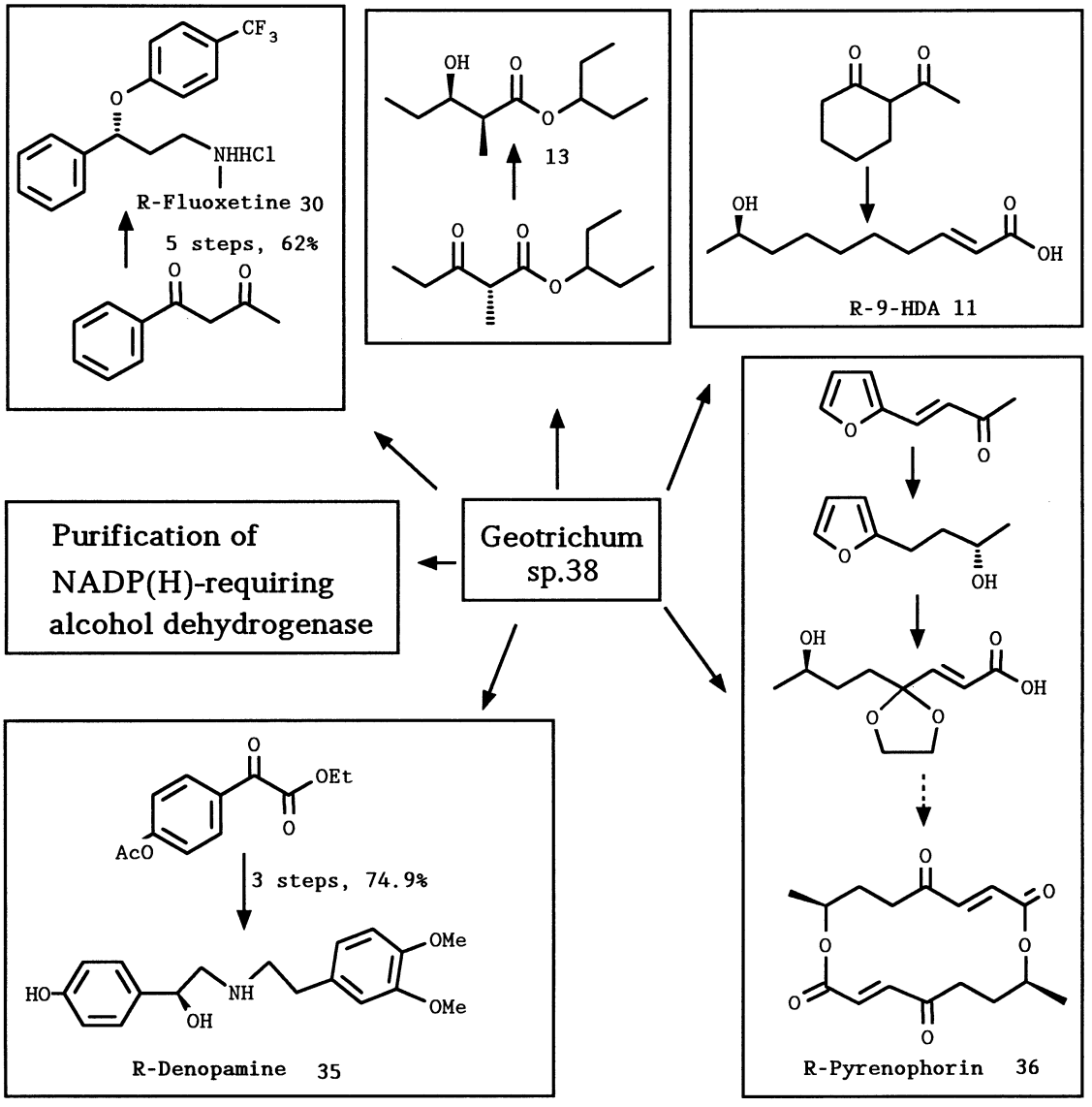


Figure 1

REFERENCES

1. H.E. Hummel, L.K. Gaston, H.H. Shorey, R.S. Kaae, K.J. Byrne and R.H.Silverstein, *Science* **181**, 873(1973).
2. Y.X. Zhu, Y.W. WU, G.Z.Guo, Z.X. Tan, G.Q. Lin, W.S. Zhou and W.G.Li, *KEXUE TONGBAO* (Special Issue) 238(1983).
3. G.Q. Lin, and Z.Y.Li, unpublished.
4. Z.X.Tan, Y.W. Wu, G.Q. Lin, B.C. Wu, H.Q. Liu, X.Q. Wang and W.S.Zhou, *Acta Chimica Sinica* (Chinese) **42,** 1178 (1984).
5. G.Q. Lin, G.Z. Guo, Y.W. Wu, B.C. Wu, L.Y. Liu, X.Q. Wang and W.S Zhou, *KEXUE TONGBAO* (Engl.) 1671 (1984).
6. Y.J. Liu, *Acta Entomol. Sinica* **22**, 90(1979).
7. X.H. Zhang, G.Z. Guo, G.Q. Lin, Y.W. Wu, P.H,Wu, Z.Y. Li and K. N. Wei, *J. Chem. Ecol.* **12,** 1263 (1986).
8. G.Z. Guo, P.H.Wu, G.Q. Lin, Z.Y. Li, X.H. Zhang and H.Q. Liu, *J. Chem. Ecol.* **16,** 819 (1990).
9. Z.X. Tan, G.Q. Lin, H.Q. Liu, G.Q. Pu, H. M. Du, J. P. Mao, L. Z. Meng and C. H. Wu, *J. Chem. Ecol.* **18**, 419(1992).
10. G. Z. Guo, H. Q. Liu, G.Q. Lin, L.Z. Meng and C.H. Wu, *YOUJIHUAXUE* 504 (1990).
11. B.A. Bierl, M. Beroza and M. Collier, *Science* **170**, 87 (1970).
12. A. Hill and W.L. Roelofs, *J. Chem. Ecol* . **8**, 383 (1982).
13. G. N. Lanier, Y.T. Qi, J.R. West, S.C. Park, F.X. Webaster and R.M. Silverstein, *J . Chem. Ecol.* 15, 1645 (1991).
14. R.R. Laurence and J.A. Pickett, *J. Chem. Soc. ,Chem. Commun.*. 59 (1982).
15. M.L. Winston, K.N. Slessor, L.G. Smirle and A.A. Kandil, *J. Chem. Ecol* . **8,** 1283 (1982).
16. L.Y. Liu and G.Q. Lin, *J. Chem. Ecol.* **16**, 1921 (1989).
17. J.K. Philips, S.F.F. Muller, J.F. Anelersen, H.M. Fales and W.E. Burkholder, *Tetrahedron Lett.* 28, 6145 (1987).
18. E. Baer and H,D.L. Fisher, *J. Biol. Chem* . **128**, 463 (1938).
19. G.Q. Lin, B.C. Wu, L.Y. Liu, X.Q. Wang and W.S. Zhou, *Acta Chimica Sinica* (Engl.) 74 (1984).
20. C.H. Wu, Personal Communication to G.Q. Lin, (1983).
21. W.S. Zhou, Z.M. Wang and G.Q. Lin, *Acta Chimica Sinica* **45**, 40 (1987).
22. G.Q. Lin, Y.Y. Jiang and W.S. Zhou, *Acta Chimica Sinica* **43**, 988 (1985).
23. G.Q. Lin, H.J. Xu, B.C. Wu, G.Z. Guo and W.S. Zhou, *Tetrahedron Lett.*, **26**, 1233 (1985).
24. Y.S. Hwang, M.S. Mulia, J.D. Chaney, G.Q. Lin and H.J. Xu, *J. Chem. Ecol.*, **13**, 245 (1987).
25. G.Q. Lin, Y.Y. Jiang, G.Z. Guo and K.M. Xia, *Acta Chimica S inica* **45**, 602(1987).
26. W.S. Zhou, J.F. Cheng and G.Q. Lin, *Acta Chimica Sinica* **46**, 274 (1987).
27. a. From Thesis of W.C. Xu , Shanghai Institute of Org. Chem., Chinese Academy of Sciences, 1992.
    b. W.R. Roush, J.A. Straub and M.S. VanNieuwenhze, *J. Org. Chem.* **56**, 1636 (1991).
28. Z.M. Wang, W.S. Zhou and G.Q. Lin, *Tetrahedron Lett* . **26**, 6221(1985).
29. S.L. Schreiber, T.S. Schreeber and D.B. Smith, *J. Am. Chem. Soc* . **109**, 1526 (1987).
30. G.Q. Lin and C.M. Zeng, *Acta Chimica Sinica* 50, 78 (1992).
31. G.Q. Lin and C.M. Zeng, *Acta Chimica Sinica* in press.
32. Part of work was published in "G.Q. Lin and C.M. Zeng, *Chinese J. of Chem.* **9**, 381 (1991)".
33. D.B. Smith, Z. Wang and S.L. Schreiber, *Tetrahedron* **46**, 4793 (1990).
34. G.Q. Ling and Z.C. Shi, unpublished.
35. From Thesis of Dr. J.X. Gu, Shanghai Inst. of Org. Chem., Chinese Academy of Sciences (1992).
36. G.Q. Lin, J.X. Gu. and Z.Y. Li, *Chinese J. of Chem.* **4,** 351 (1992).