Structure-Activity Relationship in the Gastric Cytoprotective Effect of Several Sesquiterpene Lactones

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The structural requirements for the gastric cytoprotective activity of several sesquiterpene lactones are reported. A theoretical-experimental study on the potentially active centers is carried out. The biological evaluation of reduced analogues and the simulation of the molecular interactions between these compounds and an endogenous cysteine residue suggest that the presence of a non sterically hindered Michael acceptor seems to be an essential structural requirement for the cytoprotective activity in this family of compounds. This observation suggests that cytoprotection is mediated through a Michael reaction between the sulfhydryl-containing peptides of the mucosa and Michael acceptors present in the molecules under study. This mechanism of action is in addition to and distinct from the one proposed in our previous paper, namely, that these sesquiterpenes stimulate endogenous synthesis of prostaglandins.

Introduction

The antitumor, antimicrobial, antifeedant, cytotoxic, antibacterial antifungal, allergenic contact dermatitic, and plant growth regulatory activity of several sesquiterpene lactones has been previously reported.[1-5] In our previous paper we reported them as compounds with gastric cytoprotective activity (Table I, 1-6).[6] The structure-activity relationship of these compounds has been discussed in several papers; in most of them the biological activity is attributed to the presence of α,β-unsaturated carbonyl groups. Thus, the cytotoxic, antitumor activity of several sesquiterpene lactones according to T. A. Geissman et al.[7] seems to be related to the presence of a β-unsubstituted cyclopentenone ring. On the other hand, the antimicrobial activity of these compounds appears to be independent of the presence of an α-methylene-γ-lactone moiety, as reported by K. Lee et al.[8] In our recent paper,[6] we suggest that the pharmacological activity of this family of natural products is related to the presence of an α-methylene-γ-lactone system.

According to Szabo[9] gastric cytoprotection might be mediated through at least by two different mechanisms, one concerning prostaglandins (PG) and the other involving SH-containing compounds of the mucosa. $Al(OH)_3$ was one of the first compounds reported to exhibit its protective effect via both pathways, namely, prostaglandin (PG)- and SH-containing compounds.[10] Recently, PGs and PG derivatives have been shown to prevent the formation of ulcers by a mechanism independent of their antisecretory properties[11] and to protect the gastric mucosa against lesions induced by various necrotizing agents.[12] In our previous paper[6] we demonstrated that indomethacin pretreatment resulted in a significant reduction of the cytoprotective action of dehydroleucodin and suggested that the mechanism of the protective action of this compound is related to endogenous PG production.

In view of the structural nature of the potentially active groups present in these compounds we focused our attention on the possible involvement of the SH-containing groups of the mucosa as mediators in the process of cytoprotection.[13] This mechanism would involve a Michael reaction with the Michael acceptors of the compounds under study.

In an attempt to establish the structural requirements for the gastric cytoprotective activity of these compounds, a structure-activity study was carried out. In this study, the chemical transformation and conformational study of compounds 1-5 was undertaken (Table I).

Results and Discussion

Compounds 1-4 show a common feature in their struc-

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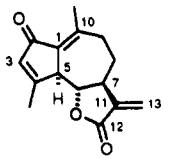
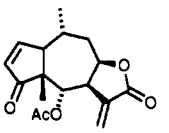
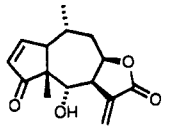
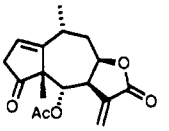
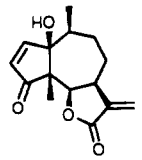
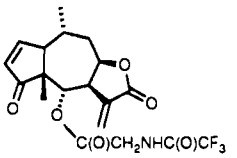
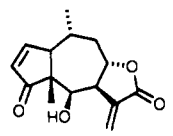
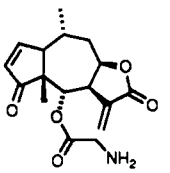
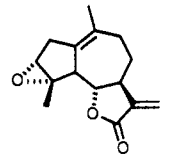
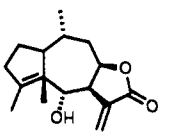
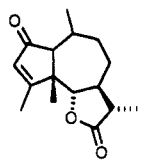
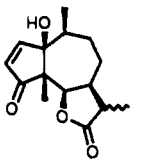
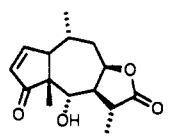
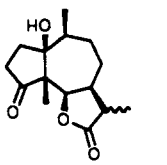
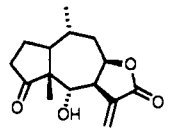
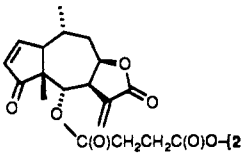
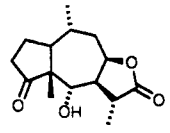
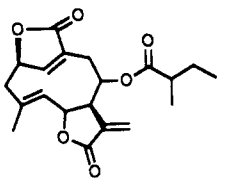
Table I. Cytoprotective Effect of Sesquiterpene Lactones at on Oral Dose of 40 mg/kg

sesquiterpene lactone		cytoprotective effect[a]	sesquiterpene lactone		cytoprotective effect[a]
compound	structure		compound	structure	
dehydroleucodin (1)		0.25 ± 0.045 (A)	helenalin 6-acetate (10)		1.00 ± 0.245 (B)
helenalin (2)		0.33 ± 0.059 (A)	mexicanin A 6-acetate (11)		0.90 ± 0.173 (B)
hymentin (3)		0.25 ± 0.035 (A)	6-*O*-[*N*-(trifluoroacetyl)glycinyl]-helenalin (12)		0.95 ± 0.11 (B)
mexicanin I (4)		0.25 ± 0.05 (A)	6-*O*-glycinylhelenalin (13)		0.85 ± 0.11 (B)
ludartin (5)		0.5 ± 0.079 (B)	guaianolide (14)		0.70 ● 0.164 (B)
11,13-dihydro-dehydroleucodin (6)		3.5 ± 0.308 (C)	11,13-dihydrohymenin (15)		4.00 ± 0.38 (C)
11,13-dihydro-helenalin (7)		0.66 ± 0.055 (B)	2,3,11,13-tetrahydrohymenin (16)		4.50 ± 0.5 (C)
2,3-dihydrohelenalin (8)		1.00 ± 0.158 (B)	helenalin succinate (17)		1.50 ± 0.35 (B)
2,3,11,13-tetrahydr-ohelenalin (9)		3.70 ± 0.21 (C)	desacylisodeoxyelephantopin 2-methylbutyrate (18)		4.00 ± 0.79 (C)

[a] See Experimental Section. Each data point represents the mean ± SD for 5 rats. The significantly different values ($p < 0.05$, ANOVA) are represented by a different letter in parentheses.

ture, an α-methylene-γ-lactone and an α,β-unsaturated cyclopentenone systems. These two functional groups have a common characteristic: both can react in principle as Michael acceptors with the SH-containing groups of the mucosa. Is is generally thought that the mechanism of the Michael additions is influenced by pH. Alkyl thiols have a pK_a's of about 10, and the pK_a's of conjugated acid enones are approximately −5. Therefore, it is likely that the reaction proceeds via a neutral Michael acceptor and deprotonated thiol under basic and neutral conditions, while under acidic conditions, a mechanism involving a protonated Michael acceptor and a neutral thiol is more reasonable. While the rumen of the stomach is strongly acidic, the putative Michael addition occurs in the mucosa where the pH is closer to neutral. Hence the reaction probably proceeds via a deprotonated thiol. An experimental–theoretical study of **1**–**5** was undertaken in order to establish their possible involvement in a Michael reaction with the SH-containing compounds of the mucosa.

Dehydroleucodin (**1**) was chosen as the first model. Compound **1** possesses the two functional groups described above and exhibits maximum cytoprotective activity. On the other hand, 11,13-dihydrodehydroleucodin (**6**) shows very weak cytoprotective activity. This fact suggests either

that the two functional groups are required for the cytoprotective activity or that the cyclopentenone cannot behave as a suitable Michael acceptor. Helenalin (2), which like 1 possesses an α-methylene-γ-lactone group and an α,β-unsaturated cyclopentenone group, also shows potent cytoprotective activity. Its 11,13-dihydro derivative (mexicanin C) (7) shows similar activity as 2. This experimental fact establishes an important difference with that observed for 6; in this case the unsaturated cyclopentenone ring seems to be a suitable Michael acceptor.

On the other hand, the 2,3-dihydrohelenalin derivative (8) shows as expected a cytoprotective activity similar to those exhibited by 2. Finally, 2,3,11,13-tetrahydrohelenalin (9) does not show any significant cytoprotective activity. The first experimental results suggest that the α-methylene-γ-lactone moiety is an effective Michael acceptor, and the second result (the lack of activity of the tetrahydro derivative) suggests that the presence of a non sterically hindered acceptor is a structural requirement for this type of pharmacological activity.

The low level of activity shown by 11,13-dihydrodehydroleucodin, in spite of the presence of two potential Michael acceptors, could be attributed to the steric hindrance produced by the methyl substituents in C-4 and C-10.[8] This steric hindrance is also observed when 1 is subjected to hydrogenation using 5% Pd on charcoal. Under these conditions and after 24 h of reaction the 11,13-dihydro derivative 6 is obtained exclusively.

Finally, the 11,13-dihydro derivative of 3, 15, does not exhibit cytoprotective activity. The same result is obtained when its 2,3,11,13-tetrahydro derivative (16) is tested. Some other derivatives of helenalin (2) have been prepared: 12, 13 and 17. The cytoprotective activity exhibited by them is in all cases lower than the cytoprotective activity shown by 2. This would be expected in view of the steric hindrance produced by the substituents on the potential Michael acceptors of the molecule.

Other derivatives of 2 in which one of the Michael acceptors have been removed 11 and 14, show a cytoprotective activity comparable to that of 2.

On the other hand, the natural product desacylisodeoxyelephantopin 2-methylbutyrate (18) does not show any significant cytoprotective activity. Again, the lack of activity shown by 18 could be attributed to the steric hindrance suffered by the two potential Michael acceptors present in the molecule.

It is interesting to note here the difference between compound 2 and compounds 1 and 3. Compound 2 possesses two active centers, and compounds 1 and 3 possess only one.

In order to explain the chemical behavior shown by the different potentially active groups of the molecules under study and to find a suitable reaction pathway, a hypothetical molecular interaction between these compounds and a potential cysteine residue of the gastric mucosa was simulated. The molecules used in this simulation and the cysteine residue were kept fixed in conformations previously obtained from MNDO calculations. The attack on the cysteine residue was simulated for all different conformations obtained previously and from different spatial orientations. The molecular interaction energetically preferred is that in which the cysteine residue is placed perpendicular to the plane of the carbon–carbon double bond. The intermolecular interactions were evaluated from 5 to 2 Å in order to include in the evaluation the possible transition-state distance, which is somewhere between 1.8 Å (the covalent bond distance) and 3.6 Å (the sum of the van der Waals radii of carbon and sulfur).

As can be seen from Figures 2, 4 and 5, when the intermolecular interactions were evaluated for the experimentally active center (C-13), the minimum of energy was reached at a interaction distance of 3 Å, with a $\Delta E < 2$ kcal/mol between 3 and 2.7 Å. This energetic increment would be overcome by the energy delivered in the process of bond formation, which is about 16 kcal/mol. On the other hand, when the intermolecular interactions were evaluated for the experimentally nonactive center (C-4), the minimum of energy was reached at a interaction distance of about 3.8 Å (larger than the sum of the van der Waals radii of carbon and sulfur). Also, a strong energetic increment is observed when the interaction distance is reduced from 3.8 to 2.7 Å (more than 20 kcal/mol). This energetic increment would also prevent the reduction of the interaction distance to the transition-state distance. The energetic increment of about 20 kcal/mol between the active and nonactive centers is large enough to explain the difficulty in the approach of the SH residue to the β-position of the β-substituted cyclopentenone ring of 1.

When the same calculations were performed on the C_1–C_{10} double bond of 1, the energetic increment found was even higher than the one found for the C_3–C_4 double bond (Figures 1 and 2). These theoretical results are in total agreement with the experimental findings presented above. The results obtained from the theoretical evaluation of the intermolecular interactions between 2 and the cysteine model (Figures 3 and 4) show that the chemical behavior of the exocyclic double bond is equivalent to that shown by 1. In the same way, the chemical behavior of the 2,3 double bond of 2 is similar to that of the exocyclic double bond. These results are also in agreement with the previous experimental study.

The theoretical evaluation of 3 shows that the chemical behavior of the exocyclic double bond of 3 is closely related to that of 1 and 2 (Figure 5). However, no conclusion can be obtained from the theoretical evaluation of the molecular interactions of the 2,3 double bond of 3 and the cysteine model. In this case some conformations are in agreement with the experimental results (no cytoprotective activity), but several other conformations show an energy gap of interaction similar to that shown by the 2,3 double bond of 2.

The lack of activity of 15 cannot be explained in terms of steric hindrance, but it could be attributed to the competition for the Michael acceptor between the SH residue of cysteine and the OH group placed on the C-1 of compound 3.

Our results would show the way in which the interaction between the SH-containing compounds and the compounds with cytoprotective activity happens; however, caution is required in such interpretation and a powerful cinetic and thermodynamic analysis will be necessary to confirm this speculation.

On the other hand, another important fact to be considered is the dependence of the cytoprotective activity of these compounds with their solubility in water. Significant differences in the solubility in water of these compounds such as those of 2 and the chlorhydrate of 13 do not produce significant changes in its pharmacological activity.

Chemistry

Selective reduction of a single double bond in systems containing two or more α,β-unsaturated carbonyl groups is a difficult synthetic operation. Several methods have been developed in order to accomplish such a goal. In most of them, selectivity is achieved by the use of modified hydride agents; in this way and in order to prepare dif-

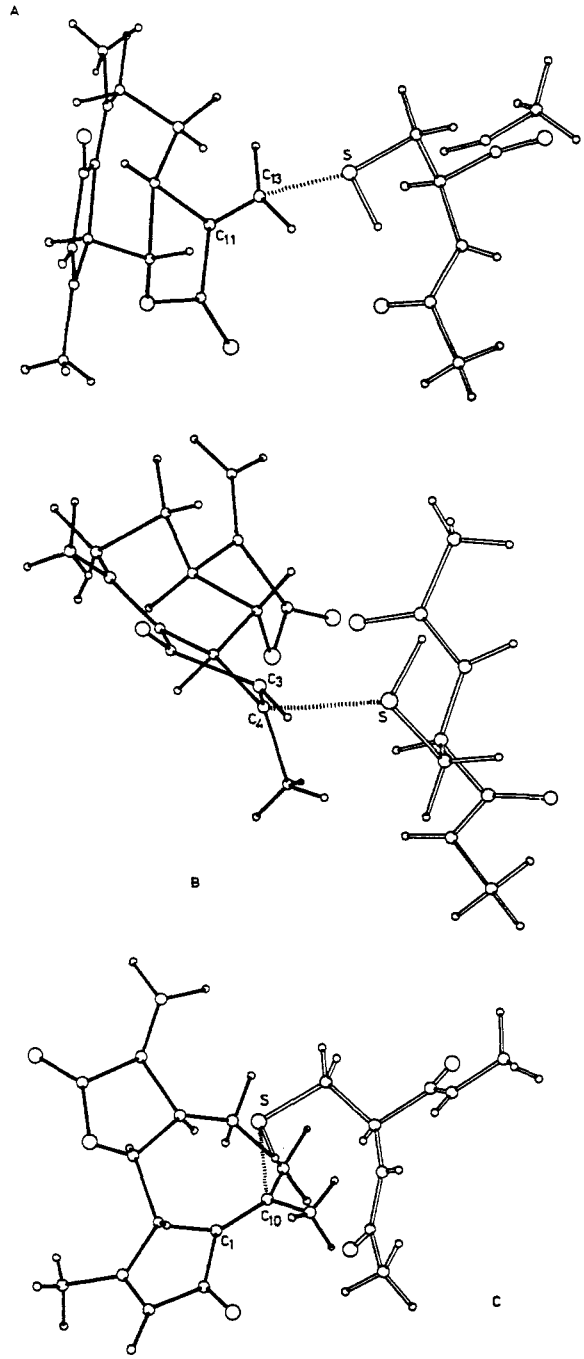


Figure 1. Spatial view of the intermolecular interactions between the chair form of dehydroleucodin (1) and a blockable cysteine residue. (A) Interaction with C_{11}–C_{13} double bond; (B) interaction with C_3–C_4 double bond; (C) interaction with C_{10}–C_1 double bond.

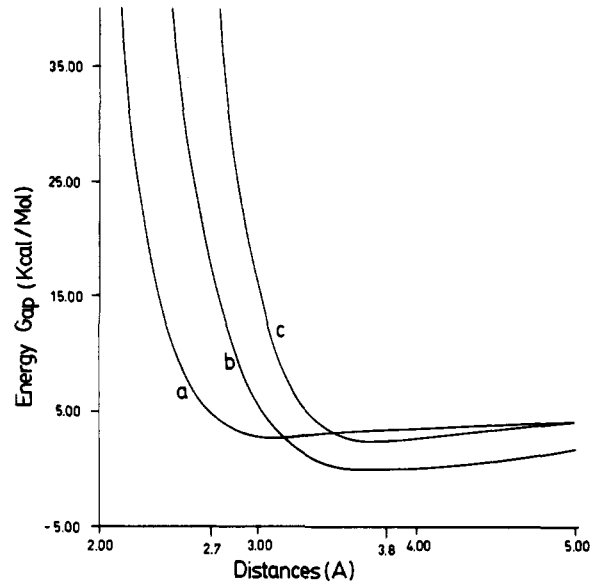


Figure 2. Energy profiles for the intermolecular interaction between the chair form of dehydroleucodin (1) and a blockable cysteine residue. (a) Cysteine–$C_{11}C_{13}$ double bond; (b) cysteine–C_3C_4 double bond; (c) cysteine–$C_{10}C_1$ double bond.

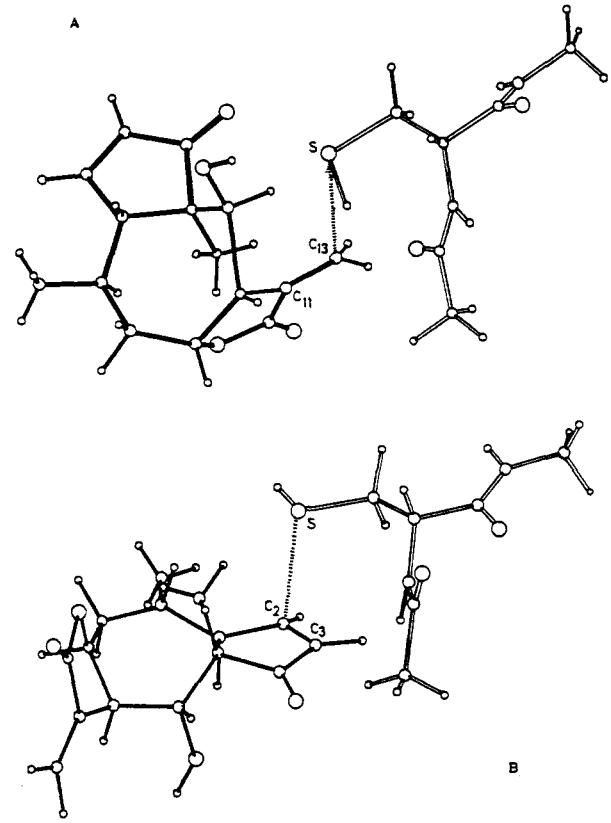


Figure 3. Spatial view of the intermolecular interactions between the chair form of helenalin (2) and a blockable cysteine residue. (A) Interaction with C_{11}–C_{13} double bond; (B) interaction with C_2–C_3 double bond.

ferent reduced derivatives of the sesquiterpene lactones **1–3**, the following procedures were carried out.

Thus, 11,13-dihydrodehydroleucodin (**6**) was obtained by reaction of (1) with $NaBH_4$ in the presence of $CoCl_2$ at −20 °C. The 11,13-dihydro derivative of **2** (mexicanin C) (**7**) was obtained in the same way.

Several reagents, namely, K-Selectride, lithium aluminum tri-*tert*-butoxyhydride, lithium aluminum hydride, and diisobutylaluminum hydride, were unsuccessfully used in order to prepare 2,3-dihydrohelenalin (**8**); however, when **2** was treated with sodium cyanoborohydride in the conditions described in the Experimental Section, the desired derivative **8** was obtained with a 76% yield. The preparation of the 2,3,11,13-tetrahydro derivative of **2**, **9**, was carried out by normal hydrogenation with 5% palladium on charcoal. Selective reduction of the 11,13 double bond of **3** was also achieved by treatment of the former with $NaBH_4$ in the presence of $CoCl_2$ at −20 °C. Finally, the 2,3,11,13-tetrahydro derivative **16** was obtained by treatment of **3** with H_2 in the presence of 5% palladium on charcoal.

Conclusion

The present study about the structure–activity relationship of sesquiterpene lactones provides experimental and theoretical evidence that the presence of a non ste-

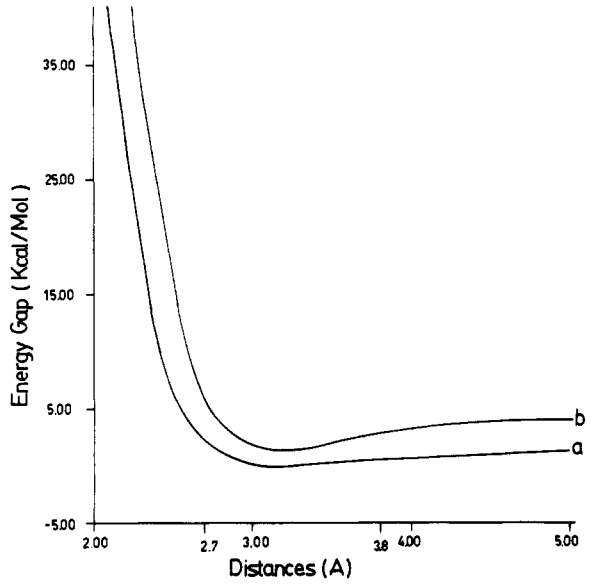


Figure 4. Energy profiles for the intermolecular interactions between the twist-chair form of helenalin (**2**) and a blockable cysteine residue. (a) Cysteine–$C_{11}C_{13}$ double bond; (b) cysteine–C_2C_3 double bond.

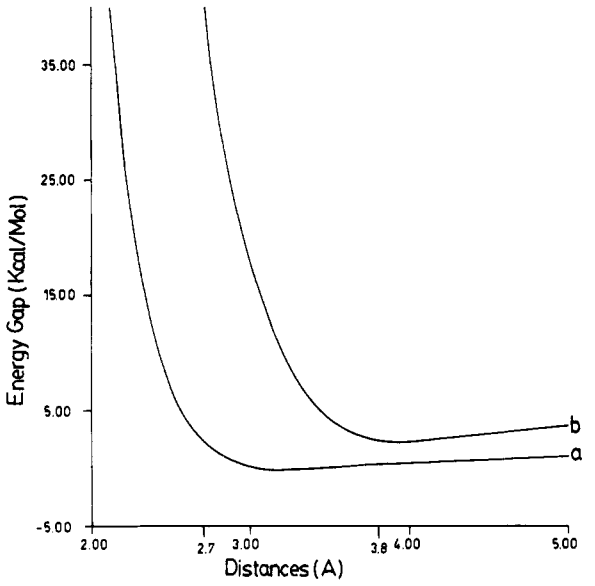


Figure 5. Energy profiles for the intermolecular interactions between the chair form of hymenin (**3**) and a blockable cysteine residue. (a) Cysteine–$C_{11}C_{13}$ double bond; (b) cysteine–C_2C_3 double bond.

rically hindered Michael acceptor seems to be an essential structural requirement for the cytoprotective activity of these compounds. The mechanism of gastric cytoprotection would be mediated, besides PG production as we suggested in our previous paper, but also by a second mechanism concerning a Michael reaction between the SH-containing compounds of the mucosa on the Michael acceptors present in the molecules under study. These results may be useful in the understanding of the structural requirements for the cytoprotective activity of sesquiterpene lactones and can provide a guide in the design of compounds with this pharmacological activity. However, further work in the subject needs to be done in order to obtain a full understanding of the mechanism of action of these compounds.

Experimental Section

General Procedures. Melting points were taken on a Leitz hot-stage apparatus and are uncorrected. Lack of melting point data indicates an indefinite melting point or noncrystallinity. Analytical thin-layer chromatography (TLC) was performed on 0.25-mm silica gel precoated plates with fluorescent indicator UV 254. Column chromatography purifications were performed on a medium-pressure chromatography system using Merck Lobar (A, B or C) silica gel columns with the help of a Fluid Metering Inc. pump. Different mixtures of EtOAc/hexanes were used as eluent.

^{13}C NMR spectra were recorded in a Bruker WP-80 spectrometer in $CDCl_3$; the ^1H NMR spectra were recorded in a Bruker WP-80 or in a Varian EM 360 A spectrometers in $CDCl_3$. Chemical shifts are reported in parts per million downfield from tetramethylsilane as internal standard. IR spectra were recorded in a Beckmann IR 10. Mass spectra were recorded on a Varian MAT 112 S spectrometer at 70 eV and 0.7 mA. For high-resolution measurements peak matching was used with an error smaller than 20 ppm and a resolution better than 6000 in the 10% valley definition.

Natural Products. All the natural products (i.e., compounds **1–5**) employed in the present study have been isolated and characterized as previously reported.[6]

Chemistry. (A) 11,13-Dihydrodehydroleucodin (6). To a solution of **1** (246 mg, 1 mmol) and $CoCl_2 \cdot 6H_2O$ (240 mg, 1 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (10 mL) at −20 °C was added $NaBH_4$ (42 mg, 1.1 mmol), and the mixture was stirred for 6 h. Then the reaction was allowed to warm to room temperature, quenched by addition of brine (25 mL), and extracted twice with CH_2Cl_2 (20 mL each). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by medium-pressure chromatography (MPC), with a 3:7 mixture of EtOAc/hexanes as eluent. Product **6** 215 mg (86%) was isolated as a white solid: mp 204–206 °C ($CHCl_3$); IR (KBr) ν 1693, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (3 H, d, J = 7 Hz, H-13), 2.35 (3 H, s, H-14), 2.42 (3 H, s, H-15), 3.55 (1 H, br s, H-6), 6.2 (1 H, br s, H-3); ^{13}C NMR ($CDCl_3$) δ C-1 (133.1), C-2 (196.3), C-3 (134.9), C-4 (168.2), C-5 (52.7), C-6 (80.2), C-7 (42.1), C-8 (25.3), C-9 (34.3), C-10 (142.7), C-11 (55.2), C-12 (177.3), C-13 (12.3), C-14 (19.8), C-15 (23.2); HRMS m/z (rel intensity) calcd for $C_{15}H_{18}O_3$ 246.3054, found 246.3048.

(B) 11,13-Dihydrohelenalin (7). To a solution of **2** (262 mg, 1 mmol) and $CoCl_2 \cdot 6H_2O$ (240 mg, 1 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (10 mL) at −20 °C was added $NaBH_4$ (42 mg, 1.1 mmol), and the mixture was treated as described for compound **6**. The residue was purified by MPC using a 4:6 mixture of EtOAc/hexanes as eluent, to give 208 mg (80%) of **7** as a white solid: mp 251–252 °C ($CHCl_3$); IR (KBr) ν 1760, 1733, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (3 H, s, H-15), 1.18 (3 H, d, J = 7 Hz, H-14), 1.35 (3 H, d, J = 7 Hz, H-13), 4.3 (1 H, m, H-6), 4.9 (1 H, m, H-8), 6.2 (1 H, dd, J = 8, 4 Hz, H-3), 7.8 (1 H, dd, J = 8, 2 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ C-1 (52.7), C-2 (161.2), C-3 (130.2), C-4 (210.5), C-5 (56.4), C-6 (71.3), C-7 (55.1), C-8 (75.8), C-9 (44.7), C-10 (28.2), C-11 (50.2), C-12 (178.2), C-13 (20.1), C-14 (19.7), C-15 (14.8); HRMS m/z (rel intensity) calcd for $C_{15}H_{20}O_4$ 264.3206, found 264.3194.

(C) 2,3-Dihydrohelenalin (8). To a solution of **2** (262 mg, 1 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (10 mL) at 25 °C was added $NaCNBH_3$ (74 mg, 1.1 mmol), and this mixture was stirred for 3 h. Then the reaction was quenched by the addition of brine (25 mL) and extracted twice with CH_2Cl_2 (20 mL each). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by MPC with a 4:6 mixture of EtOAc/hexanes as eluent to give 202 mg (76%) of product **8** as a white solid: mp 232–235 °C ($CHCl_3$); IR (KBr) ν 1780, 1733, 1648 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.28 (3 H, s, H-15), 1.2 (3 H, d, J = 8 Hz, H-14), 3.5 (1 H, m, H-1), 4.2 (1 H, m, H-6), 4.8 (1 H, m, H-8), 5.9 (1 H, d, J = 4 Hz, H-13), 6.5 (1 H, d, J = 4 Hz, H-13'); ^{13}C NMR ($CDCl_3$) δ C-1 (48.2), C-2 (24.6), C-3 (33.9), C-4 (218.3), C-5 (54.3), C-6 (72.2), C-7 (57.4), C-8 (79.8), C-9 (42.4), C-10 (27.9), C-11 (134.7), C-12 (169.7), C-13 (121.9), C-14 (14.7), C-15 (19.8); HRMS m/z (rel intensity) calcd for $C_{15}H_{20}O_4$ 264.3206, found 264.3217.

(D) 2,3,11,13-Tetrahydrohelenalin (9). To a solution of **2** (262 mg, 1 mmol) in a 1:1 mixture of EtOAc/MeOH was added 5% Pd/charcoal, and the mixture was stirred at room temperature under H_2 atmosphere (2 atm) for 24 h. Then the mixture was filtered and the solvent removed in vacuo. The residue was

purified by MPC with a 3.5:6.5 mixture of EtOAc/hexanes as eluent to give 135 mg (57%) of product **9** and a byproduct [78 mg (25%)] whose structure remains unclear: mp 210–212 °C ($CHCl_3$); IR (KBr) ν 1780, 1750, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (3 H, s, H-14), 1.08 (3 H, d, J = 7 Hz, H-15), 1.33 (3 H, d, J = 7 Hz, H-13), 4.4 (1 H, br s, H-6), 5.0 (1 H, m, H-8); ^{13}C NMR ($CDCl_3$) δ C-1 (47.3), C-2 (24.3), C-3 (35.1), C-4 (217.7), C-5 (54.3), C-6 (69.8), C-7 (53.6), C-8 (76.7), C-9 (44.9), C-10 (29.5), C-11 (50.2), C-12 (176.2), C-13 (19.8), C-14 (15.8), C-15 (17.3); HRMS m/z (rel intensity) calcd for $C_{15}H_{22}O_4$ 266.3364, found 266.3358.

(E) Helenalin 6-Acetate (10). To a solution of **2** (262 mg, 1 mmol) in CH_2Cl_2 (10 mL) were added Et_3N (3 mL), (dimethylamino)pyridine (DMAP) (13 mg), and acetic anhydride (2 mL), and the solution was stirred for 3 h at 25 °C. Then the reaction mixture was quenched by the addition of water and extracted twice with CH_2Cl_2 (20 mL each). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue obtained was purified by MPC with a 3:7 mixture of EtOAc/hexanes as eluent to give 285 mg (94%) of product **10** as a white solid: IR (KBr) ν 1760, 1730, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (3 H, s, H-15), 1.28 (3 H, d, J = 7 Hz, H-14), 1.98 (3 H, s, $C(O)CH_3$), 4.72 (1 H, m, H-8), 5.5 (1 H, br s, H-6), 6.15 (1 H, dd, J = 8, 4 Hz, H-3), 6.20 (1 H, d, J = 4 Hz, H-13), 6.5 (1 H, d, J = 4 Hz, H-13′), 7.74 (1 H, dd, J = 8, 2 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ C-1 (52.8), C-2 (162.3), C-3 (129.1), C-4 (211.4), C-5 (57.4), C-6 (74.7), C-7 (59.9), C-8 (80.7), C-9 (41.7), C-10 (27.4), C-11 (135.4), C-12 (169.8), C-13 (123.1), C-14 (20.1), C-15 (19.8), C-16 (201.3), CH_3 (16.9); HRMS m/z (rel intensity) calcd for $C_{17}H_{20}O_5$ 304.3420, found 304.3411.

(F) Mexicanin A 6-Acetate (11). To a solution of acetate **10** (456 mg, 1.5 mmol) in dry C_6H_6 (15 mL) was added H_2SO_4 (c) (0.1 mL), and the mixture was refluxed for 16 h. Then the solution was refrigerated in an ice bath, neutralized with $NaHCO_3$, and extracted twice with CH_2Cl_2 (20 mL each). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by MPC with a 3:7 mixture of EtOAc/hexanes as eluent to give 227 mg (50%) of **11** as a white solid and 135 mg (30%) of starting material back: mp 181–183 °C ($CHCl_3$); IR (KBr) ν 1765, 1718, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (3 H, s, H-15), 1.3 (3 H, d, J = 7 Hz, H-14), 1.98 (3 H, s, $C(O)CH_3$), 4.85 (1 H, m, H-8), 5.25 (1 H, d, J = 6 Hz, H-2), 5.9 (1 H, d, J = 4 Hz, H-13), 6.2 (1 H, d, J = 4 Hz, H-13′); ^{13}C NMR ($CDCl_3$) δ C-1 (154.3), C-2 (121.9), C-3 (39.5), C-4 (215.9), C-5 (54.7), C-6 (78.9), C-7 (60.1), C-8 (73.7), C-9 (40.9), C-10 (29.5), C-11 (135.3), C-12 (174.1), C-13 (124.7), C-14 (18.4), C-15 (17.4), C-16 (202.1), CH_3 (19.3); HRMS m/z (rel intensity) calcd for $C_{17}H_{20}O_5$ 304.3420, found 304.3431.

(G) *N*-(Trifluoroacetyl)glycine. Glycine (375 mg, 5 mmol) was placed in a two-neck round-bottom flask equipped with condenser. The solid was refrigerated in an ice bath. Then, trifluoroacetic anhydride (1.4 mL, 10 mmol) was added and the mixture stirred by 1 h at 0 °C. Then the mixture was heated to 80 °C and stirred an additional 3 h. Then the solution was cooled, and the solid obtained was filtrated and washed with cooled C_6H_6. Crystallization from hot C_6H_6/EtOAc (7:3) yielded 700 mg (82%) of *N*-(trifluoroacetyl)glycine, mp 120–122 °C.

(H) 6-*O*-[*N*-(Trifluoroacetyl)glycinyl]helenalin (12). To a solution of **2** (262 mg, 1 mmol) and *N*-(trifluoroacetyl)glycine (171 mg, 1 mmol) in dry CH_2Cl_2 (20 mL) at 25 °C were added dicyclohexylcarbodiimide (DCC) (226 mg, 1 mmol) and DMAP (20 mg), and the solution was stirred for 5 h. Then the reaction was quenched by the addition of brine and extracted twice with CH_2Cl_2 (20 mL each). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue obtained was purified by MPC with a 4:6 mixture of EtOAc/hexanes as eluent to give 117 mg (28%) of product **12**: IR (KBr) ν 3340, 1765, 1750, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (3 H, s, H-15), 1.25 (3 H, d, J = 7 Hz, H-14), 2.8 (1 H, m, H-1), 3.5 (1 H, m, H-7), 4.0 (2 H, d, J = 6 Hz, $NHCH_2C(O)OR$), 4.9 (1 H, m, H-8), 5.4 (1 H, s, H-6), 6.1 (1 H, dd, J = 4, 8 Hz, H-3), 6.2 (1 H, d, J = 4 Hz, H-13), 6.5 (1 H, d, J = 4 Hz, H-13′), 7.6 (1 H, br s, N-H), 7.75 (1 H, dd, J = 8, 2 Hz, H-2); HRMS m/z (rel intensity) calcd for $C_{19}H_{20}F_3NO_6$ 415.3686, found 415.3694.

(I) 6-*O*-Glycinylhelenalin (13). A total of 262 mg (1 mmol) of **2** was dissolved in a 7% K_2CO_3 solution in MeOH/H_2O (2:1), and the solution was stirred at room temperature for 2 h. Then the solution was neutralized to pH = 6 with acetic acid and extracted twice with CH_2Cl_2 (20 mL each). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by MPC with a 5.5:4.5 mixture of EtOAc/hexanes as eluent to give 193 mg (46%) of product **13**: IR (KBr) ν 3350, 1765, 1740, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (3 H, s, H-15), 1.22 (3 H, d, J = 7 Hz, H-14), 2.72 (1 H, m, H-1), 3.52 (1 H, m, H-7), 3.98 (2 H, d, J = 6 Hz, $NH_2CH_2C(O)OR$), 4.86 (1 H, m, H-8), 5.35 (1 H, s, H-6), 6.1 (1 H, dd, J = 4, 8 Hz, H-3), 6.2 (1 H, d, J = 4 Hz, H-13); 6.5 (1 H, d, J = 4 Hz, H-13′), 7.6 (1 H, br s, N-H), 7.75 (1 H, dd, J = 8, 2 Hz); HRMS m/z (rel intensity) calcd for $C_{17}H_{21}NO_5$ 319.3569, found 319.3562.

(J) Guaianolide (14). To a solution of **2** (524 mg, 2 mmol) in dry THF (10 mL) at 0 °C was added $LiAl(O\text{-}t\text{-}Bu)_3H$ (560 mg, 2.2 mmol), and the mixture was stirred for 2 h. Then the reaction was allowed to warm to room temperature and was stirred an additional 3 h. Then the reaction was quenched by addition of brine (25 mL) and extracted twice with CH_2Cl_2 (20 mL each). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by MPC with a 3:7 mixture of EtOAc/hexanes as eluent to give 170 mg (35%) of guaianolide (**14**) and 160 mg (30%) of 2,3-dihydrohelenalin (**8**): IR (KBr) ν 3500, 1760, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (3 H, d, J = 7 Hz, H-14), 1.72 (3 H, d, J = 1, 5 Hz), 4.4 (1 H, m, H-6), 5.0 (1 H, m, H-8), 5.8 (1 H, d, J = 4 Hz, H-13), 6.4 (1 H, d, J = 4 Hz, H-13′); HRMS m/z (rel intensity) calcd for $C_{15}H_{20}O_3$ 248.3212, found 248.3205.

(K) 11,13-Dihydrohymenin (15). To a solution of **3** (262 mg, 1 mmol) and $CoCl_2 \cdot 6H_2O$ (240 mg, 1 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (10 mL) at −20 °C was added $NaBH_4$ (42 mg, 1.1 mmol), and the mixture was treated as decribed for compound **6**. The residue was purified by MPC with a 3:7 mixture of EtOAc/hexanes as eluent to give 198 mg (75%) of product **15**: IR (KBr) ν 3337, 1780, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03 (3 H, s, H-15), 1.08 (3 H, d, J = 7 Hz, H-14), 1.15 (3 H, d, J = 7 Hz, H-13), 4.7 (1 H, m, H-6), 6.2 (1 H, d, J = 6 Hz, H-3), 7.6 (1 H, d, J = 6 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ C-1 (80.9), C-2 (162.4), C-3 (131.2), C-4 (208.7), C-5 (58.3), C-6 (76.8), C-7 (42.4), C-8 (28.3), C-9 (29.1), C-10 (42.2), C-11 (54.9), C-12 (175.3), C-13 (13.2), C-14 (16.5), C-15 (18.5); HRMS m/z (rel intensity) calcd for $C_{15}H_{20}O_4$ 264.3206, found 264.3202.

(L) 2,3,11,13-Tetrahydrohymenin (16). To a solution of **3** (262 mg, 1 mmol) in a 1:1 mixture of EtOAcMeOH was added 5% Pd/C (20 mg), and the mixture was stirred at room temperature under H_2 atmosphere (2 atm) for 24 h. Then the mixture was filtered and the solvent removed in vacuo. The residue was purified by MPC with a 4:6 mixture of EtOAc/hexanes as eluent to give 235 mg (90%) of product **16**: IR (KBr) ν 3370, 1760, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (3 H, s, H-15), 1.02 (3 H, d, J = 7 Hz, H-14), 1.17 (3 H, d, J = 7 Hz, H-13), 4.83 (1 H, m, H-6); ^{13}C NMR ($CDCl_3$) δ C-1 (81.1), C-2 (32.2), C-3 (34.3), C-4 (215.7), C-5 (57.2), C-6 (78.1), C-7 (44.7), C-8 (26.9), C-9 (31.2), C-10 (43.1), C-11 (53.2), C-12 (174.2), C-13 (14.1), C-14 (15.2), C-15 (17.3); HRMS m/z (rel intensity) calcd for $C_{15}H_{22}O_4$ 266.3364, found 266.3358.

(M) Helenalin Succinate (17). To a solution of **2** (524 mg, 2 mmol) and succinic acid (118 mg, 1 mmol) in dry CH_2Cl_2 (20 mL) were added dicyclohexylcarbodiimide (DCC) (453 mg, 1 mmol) and (dimethylamino)pyridine (DMAP) (15 mg), and the mixture was stirred overnight at room temperature. Then the reaction was quenched by addition of brine (50 mL) and extracted twice with CH_2Cl_2 (25 mL each). The organic layers were combined and dried over Na_2SO_4, end the solvent was removed in vacuo. The residue was purified by MPC with a 3:7 mixture of EtOAc/hexanes as eluent to give 485 mg (80%) of product **17**: IR (KBr) ν 1754, 1720, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (6 H, s, H-15), 1.22 (6 H, d, J = 7 Hz, H-14), 2.7 (4 H, s, $OC(O)\text{-}CH_2CH_2C(O)O$), 5.0 (2 H, m, H-8), 5.45 (2 H, s, H-6), 6.2 (4 H, m, H-3, H-13), 6.55 (2 H, d, J = 4 Hz, H-13′), 7.8 (2 H, dd, J = 7, 2 Hz, H-2); HRMS m/z (rel intensity) calcd for $C_{34}H_{38}O_{10}$ 606.6682, found 606.6697.

Induction of Gastric Lesions. Gastric lesions were produced according to the method of Robert et al.[22] Male Wistar rats weighing ca. 180 g were fasted for 24 h and deprived of water for 19 h prior to the experiments. All rats were housed in wire

mesh-bottom cages throughout the study to prevent coprophagy. Absolute EtOH (1 mL) administered orally was employed as the necrotizing agent, and 1 h later the animals were decapitated. The stomachs were removed, opened along the greater curvature, and washed gently with ice-cold saline solution. The degree of erosion in the glandular part of stomach was assessed from a scoring system designed by Merazzi-Uberti and Turba,[24] as follows: 0, no erosions; 1, 1–3 small erosions (4-mm diameter or smaller); 2, more than 3 small erosions or 1 large erosion; 3, 1 large erosion and more than 3 small erosions; 4, 3–4 large erosions; 5, any very large erosion or ulcer perforation.

The results were expressed in terms of an ulcer factor which is the average severity of erosions per rat for each group on the scale from 0 to 5. The sum of these values was divided by the number of animals. The control rats treated with absolute EtOH showed an average score of 4.8. The control rats without treatment showed an average score of 0.0. The drugs tested in this study were prepared just before the experiment as follows: Compounds 1–17 were suspended in water. The control rats (group 1) were given 1 mL of absolute EtOH. Compounds 1–17 (40 mg/kg) were given 1 h before the oral administration of EtOH (group 2).

Calculation Methods. The conformational study of the molecules was performed using the molecular orbital (MO) MNDO method[14] and a potential atom–atom empirical method. A complete optimization of the geometrical parameters for the different conformations of the molecules under study was made. The starting geometrical parameters (bond angles and distances) were taken from X-ray data of structurally related compounds.[15]

In order to evaluate the possible intermolecular interactions between the compounds under study with potential receptors, these interactions were simulated under molecular mechanisms calculations. The calculations were performed using a potential atom–atom empirical method[16] designed for our group. This method has been used previously for other biological systems with satisfactory results.[17–19] In this method the energy of the system (E) is calculated as the sum of the following contributions: $E = E_{nb} + E_{solv}$, were E_{nb} is the sum of potential energies due to interactions between pairs of nonbonded atoms and E_{solv} is the solvation energy of the molecules in solution.

For the calculation of potential energy corresponding to the interaction between pairs of nonbonded atoms, the function proposed by Hill[20] was used. It introduces only two parameters: α, which depends only on the distance, and ϵ, which is an energy parameter:

$$E_{ij(nb)} = \{-2.25\alpha_{ij}^{-6} + (8.28 \times 10^5)\exp(-\alpha_{ij}/0.0736)\}\epsilon_{ij}$$

with

$$\alpha_{ij} = r_{ij}(r^*_i + r^*_j)$$

where $E_{ij(nb)}$ = the potential energy of the interaction between the nonbonded atoms i and j in kcal/mol, r_{ij} = the interatomic distance of atoms i and j in Å, r^*_i and r^*_j = van der Waals radii of atoms i and j in Å, and ϵ_{ij} = energy parameters for atoms i and j in kcal/mol. E_{nb} is expressed in kcal/mol, and the parameters used for the calculations are those recommended by Allinger and co-workers.[21] For the calculations, only the intramolecular interactions between both molecules were evaluated. The intramolecular interactions of each molecule were not taken into account.

The second term takes into account the solvent effect using a solvent continuum model. In this model we consider the molecule as a set of point charges immersed in a continuum dielectric. Thus the solvent effect results as the sum of two contributions: (a) the solvent effect on each atom using the model proposed by Stokes,[22] and (b) the sum of the interactions between pairs of atoms using the model proposed by Hoijtink et al.[23] For the calculations, the atoms are considered to be elipsoidal and the effective radius is assumed as the average of both covalent and van der Waals radiis, the effective dielectric constant (D_{eff}) was considered as $D^{1/2}$, and the diameter of water molecules was assumed as 2.86 Å:

$$E_{solv} = -\{\sum_i (q_i^2/2)[1/r_i - 2.86/(r_i + 2.86)D_{eff} - 1/(r_i + 2.86)D]\} + \sum_{i<j}(q_iq_j/r_{ij})(1 - 1/D_{eff})$$

where q_i and q_j = the atomic charges of atoms i and j, r_i = the average diameter of atom i, D = the macroscopic dielectric constant, and D_{eff} = the effective dielectric constant.

The molecular mechanical calculations were carried out in the neutral form of the thiol since the errors caused by this type of substitution may be less severe than those involved with gas-phase molecular mechanics calculations of charged species.

Michael additions are fairly exothermic processes (the heat of enthalpy calculated at the ab initio 3-21G level for the reaction of ethane thiol with cyclopentenone is 19.3 kcal/mol). Applying Hammond's postulate, it is reasonable that the transition state for these reactions are reactant-like. Hence it is legitimate to use ground-state geometries of the reactants to model the transition state.

Acknowledgment. We thank M. G. Sierra (IQUIOS) Rosario for the ^{13}C NMR and Mr. M. Soloa for his technical assistance. This work was supported by a grant of Fundación Antorchas and CONICET (Argentina).

Registry No. 1, 36150-07-9; 2, 6754-13-8; 3, 20555-04-8; 4, 5945-41-5; 5, 36149-87-8; 6, 17946-87-1; 7, 141434-04-0; 8, 35412-73-8; 9, 10180-96-8; 10, 10180-86-6; 11, 106292-57-3; 12, 141344-85-6; 13, 141344-86-7; 14, 141344-87-8; 15, 141344-88-9; 16, 28582-84-5; 17, 141344-89-0; 18, 113626-23-6; *N*-trifluoroacetylglycine, 383-70-0; glycine, 56-40-6.

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