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Recent discoveries in the chemistry of natural products

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Abstract: Phytochemical investigations on the medicinal plants of Turkish and Pakistani origin have resulted in the isolation of several new natural products. *Buxus sempervirens* and *Fritillaria persica* of Turkish origin have yieled several new steroidal alkaloids. New withanolides and furanoid diterpenoids were isolated from *Withania somnifera* and *Tinospora malabarica* of Pakistani origin. Chemical transformations of catharanthine and leurosine into vinblastine and its analogues was also achieved. The CD *in situ* complexation method has been developed as a tool for the determination of absolute configurations of Cottonogenic derivatives.

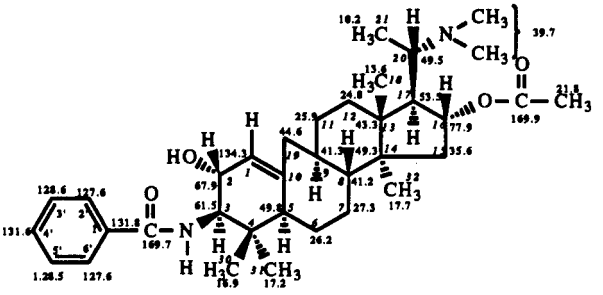
(+)-Semperviraminol (1): A New Triterpenoidal Alkaloid from the Leaves of *Buxus sempervirens* of Turkish Origin

Buxus sempervirens L. (Buxaceae) is widely distributed throughout Eurasia, North, South and Central America (1). The aqueous extracts of the plant are widely used in the treatment of a variety of diseases such as malaria, tuberculosis, rheumatism and other skin infections in folk medicine (2). The phytochemical investigations on the ethanolic extract of the roots of *B. sempervirens* have yielded several new steroidal alkaloids.

(+)-Semperviraminol (**1**), a new triterpenoidal alkaloid, was isolated from the ethanolic extract of the roots of *B. sempervirens* as a colourless amorphous solid. The UV spectrum of the compound **1** showed maximum absorption at 226 nm indicating the presence of a secondary benzamide chromophore in the molecule (3). The IR spectrum displayed intense absorptions at 3416 (OH), 3310 (NH), 1709 (ester carbonyl), 1645 (α,β-unsaturated amide carbonyl) and 1595 (C=C) cm^{-1}. The HREI mass spectrum of **1** showed the molecular ion peak at *m/z* 564.7865 which is in agreement with the molecular formula $C_{35}H_{52}N_2O_4$ and indicated the presence of eleven degrees of unsaturation in the molecule. The ion at *m/z* 539.2612 was due to the loss of a methyl group from the molecular ion. The peak at *m/z* 105.0409 was due to the benzoyl cation, while the ion at *m/z* 175.2345 arose by the *retro* Diels-Alder cleavage of ring A and suggested the presence of a double bond in the ring A. The base peak at *m/z* 72 was due to trimethylimminium cation, whereas the ion at *m/z* 157.0467 was due to the cleavage of ring D along with the attached substituents.

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of **1** featured four 3H sharp singlets at δ 0.85, 0.91, 0.94 and 1.22 due to the four tertiary methyl protons. A 3H doublet at δ 1.29 ($J_{21,20}$= 6.5 Hz) was due to a C -21 secondary methyl protons. A 3H singlet at δ 2.06 was attributed to the acetyl methyl protons. A 6H broad singlet at δ 2.27 was a characteristic of the *N*, *N*-dimethyl protons. The allylic C-19 methylene protons resonated at δ 2.64 ($J_{19\beta,19\alpha}$= 13.8 Hz, $J_{19\alpha,9\alpha}$= 4.4 Hz). A 1H double doublet at δ 3.95 ($J_{3\alpha,2\beta}$= 9.7 Hz, $J_{3\alpha}$, NH= 9.5 Hz), while the C -2 methine proton appeared as a broad doublet at δ 4.00 ($J_{2\beta,3\alpha}$= 9.7 Hz). The α-stereochemistry of the C-2/OH group was established on the basis of H-2/H-3 coupling constant. The coupling constant (9.7 Hz) represents *trans* diaxial coupling between the C-2 and C-3 protons. A study of the Dreiding models of **1** showed that ring A exists in a chair conformation in which the C-3/N and C-2/O bonds are equatorially oriented. The C-16 methine proton, geminal to the acetoxy group, resonated at δ 4.77, the C-1 olefinic proton appeared at δ 5.69, whereas the amidic NH resonated at δ 6.14 ($J_{NH,3\alpha}$= 9.3 Hz). The 2H and 3H multiplets at δ 7.65 and 7.30 were due to the aromatic protons and represented the monosubstituted phenyl moiety. COSY-45° and HOHAHA spectra revealed four isolated spin systems in the molecule (4, 5).

The ^{13}C-NMR spectrum of **1** showed the resonance of all 35 carbon atoms and is presented around struture **1**.The inverse NMR experiments (HMQC and HMBC) helped to establish the direct $^{1}H/^{13}C$ one-bond and long range correlations, respectively. On the basis of these spectroscopic studies structure **1** was established for this new triterpenoidal base.



1

Persinin (2): A New Steroidal Alkaloid from *Fritillaria persica*

Fritillaria persica (Liliaceae) is abundantly found in Turkey. The plants of genus *Fritillaria* are extensively used in the indigenous system of medicine for the treatment of various diseases (6). The phyto-chemical studies on the ethanolic extract of the bark of *F. persica* have resulted in the isolation of persinin (**2**), a new steroidal alkaloid as a colorless gummy material. The UV spectrum of **2** showed terminal absorption, indicating the lack of any chromophore. The IR spectrum afforded absorptions at 3350 (OH), 1638 (six-membered ketonic group) and 2860 (*trans*-quinolizidine moiety) cm^{-1}.

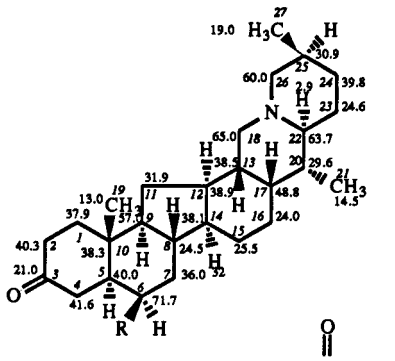
The EIMS of **2** showed molecular ion peak at *m/z* 413 which corresponded to the molecular formula $C_{37}H_{43}NO_2$ and indicated the presence of seven degrees of unsaturation in the molecule. Other major fragments at *m/z* 412 (M^+-H), 398 (M-CH_3), 357 (M^+-C_4H_8) were also observed in the mass spectrum. The compound **2** showed the base peak at *m/z* 111, which is characteristic of cevine-type bases (7).

The ^{1}H-NMR spectrum of **2** manifested signals representing two secondary methyl groups *i.e.* CH_3-27 (δ 0.96, J= 6.2 Hz) and CH_3-21 (δ 1.37, J= 7.4 Hz) respectively. A three-proton singlet at δ 1.23 was assigned to the C-19 methylene protons. A double doublet at δ 3.85 (J_1= 5.4 Hz, J_2= 2.8 Hz) was assigned to the C-6α proton, geminal to the hydroxyl group. The coupling constants (J_1= 5.4 Hz, J_2= 2.8 Hz) indicated the equatorial orientation of the C-6 methine proton (5). A 2H broad doublet at δ 3.16 (J= 6.6 Hz) was ascribed to the C-18 methylene protons vicinal to the nitrogen atom.

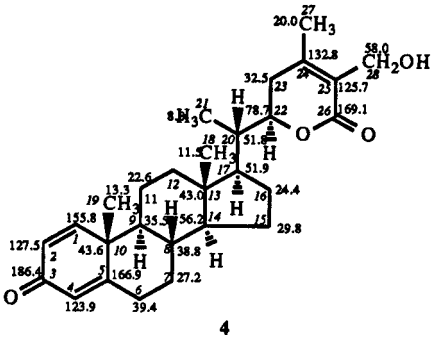
The COSY-45° spectrum of **2** showed cross-peaks between the C-27 methyl protons (δ 0.96) and C-25 methine proton (δ 1.98) which was further coupled with the C-26 methylene C-24 protons (δ 2.85 and 2.77). The C-21 methyl protons showed coupling with the C-20β proton (δ 1.90). Cross-peaks between the C-18 methylene protons (δ 3.16) and the C-13β (δ 2.1) and C-17 (δ 1.56) protons were also observed in the spectrum. The C-6α proton (δ 3.85) showed coupling with the C-5 protons (δ 1.99).

The ^{13}C-NMR spectrum showed the resonances of all 37 carbon atoms and is shown around structure **2**. Direct $^{1}H/^{13}C$ connectivities were determined by the HMQC spectrum.

The presence of a hydroxyl group at C -6 in persinin (**2**) was further confirmed by acetylation. The EIMS of 6-acetyl persinine (**3**) showed M^+ at *m/z* 455 ($C_{39}H_{46}NO_3$). The ^{1}H-NMR spectrum of the acetylated product **3** showed a downfield shift in the C-6 proton from δ 3.85 to δ 4.67 as well as an additional signal of the acetyl methyl protons at δ 2.34. The rest of the ^{1}H -NMR spectrum was similar to that of the parent alcohol. Based on these studies, structure **2** was assigned to this new steroidal alkaloid.



2 R = OH ; 3 R = OC - CH_3



4

Withasomidienone (4): A New Withanolide from *Withania somnifera*

Withania somnifera Dun. (Solanaceae), a perennial plant, is widely distributed along the shores of the Mediterranean sea, as well as in India, South Africa, Pakistan and some other countries (7). Various therapeutic properties have been attributed to this plant and it has been used in the indigenous system of medicine for the treatment of ulcers, rheumatism, cough, dropsy, consumption and senile debility (8).

Withasomidienone (4) $C_{28}H_{38}O_4$ (*m/z* 438) was isolated from the methanolic extract of the *W. somnifera*. The UV spectrum exhibited aborption at 234 nm, indicating the presence of a conjugated cyclohexadienone system (9). The IR spectrum afforded intense absorptions at 3550 (OH), 1650 (α,β-unsaturated ketone) and 1615 (C = C) cm^{-1}.

The ^{1}H-NMR spectrum of **4** revealed the presence of three tertiary methyls as 3H singlets at δ 0.77, 1.22 and 2.03. A doublet at δ 1.03 ($J_{21,20}$ = 6.6 Hz) was assigned to the C-21 secondary methyl protons. An AB doublet at δ 4.35 and 4.37 ($J_{27,27'}$= 12.6Hz) was due to the C-27 hydroxymethylene protons. A downfield broad doublet at δ 4.39 (J= 13.2 Hz) was assigned to the C -22 methine proton of the lactone moiety. Two olefinic signals resonated as a doublet at δ 7.05 ($J_{1,2}$= 10.1 Hz) and as a double doublet at δ 6.23 ($J_{2,1}$= 10.1 Hz, $J_{2,4}$ =1.9 Hz) due to the C-1 and C-2 vinylic protons. Another broad singlet at δ 6.06 was due to the C-4 vinylic proton. These observations supported a C-3 ketone function in conjugation with the C-2/C-1 and C-4/C-5 double bonds.

The COSY-45° spectrum of **4** revealed many important homonuclear connctivities. For instance, the Me-21 doublet (δ 1.03) showed a strong cross-peak with a one-proton multiplet at δ 2.50, assigned to a methine proton (H-20), which was further coupled to the downfield H-22 methine (δ 4.39) in the COSY 45° spectrum. The methine H-22 (δ 4.39) was in turn coupled with the methylene H-22 resonating at δ 2.00. Coupling between the vinylic H-2 and H-4 was also observed in the spectum. The long-range ^{1}H-^{1}H connectivities were determined by recording a series of HOHAHA spectra with variable delays (100, 60, 20 msec).H-1 (δ 7.05) showed long-range interaction with vinylic H-4, while H-4 exhibited couplings with the vinylic H-2 as well as with the allylic H-6 methylene protons of ring B. The Me-21 protons (δ 1.03) displayed a cross-peak with H-22 (δ 4.39) and H-23 (δ 2.00), whereas the methine H-20 (δ 2.50) also showed cross-peaks with H-23. On the other hand the methylene H_2-23 displayed long-range interactions with H-20, H-21, and H-28. The hydroxymethylene protons (H-27) also exhibited homalllylic coupling with the allylic CH_3-28.

The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) of **4** showed the resonances of all twenty eight carbon atoms and is shown around structure **3**.The one-bond ^{1}H/^{13}C correlations for **4** were determined on the basis of HMQC experiments. The long-range ^{1}H/^{13}C interactions of **4** were determined by HMBC experiments.

Menispermacide (5): A Novel Furanoid Diterpenoid from *Tinospora malabarica*

Tinospora malabarica Meirs (Menispermaceae) is cultivated throughout Pakistan. The aqueous extract of the plant is used in the indigenous system of medicine for the treatment of intermittent fever, liver, and eye ailments and is reputed to be a tissue builder and emetic (10).

Menispermacide (**5**) was isolated from *T. malabarica* and exhibited UV absorption at 206 nm. The IR spectrum displayed intense absorptions at 1730 (lactone carbonyl), 1720 (ketonic carbonyl), and 880 (furan ring) cm^{-1}. The highest mass peak in the EIMS of **5** was *m/z* 359 corresponding to the formula $C_{20}H_{23}O_6$ which was not in agreement with the ^{13}C-NMR spectra (DEPT and BB decoupled) which had 21 carbon resonances. Similarly the ^1H-NMR spectrum of **5** also showed more protons than expected on the basis of the mass spectrum.

The structure of **5** was therefore unambiguously established by single crystal X-ray diffraction technique. Compound **5** was recrystallized from MeOH-CH_2Cl_2. Crystals formed in the orthorhombic space group $P2_12_12_1$ with a = 10.155(3), b = 11.214(4), c = 18.762(8) Å and one molecule of composition $C_{21}H_{26}O_6S_2$ forming the asymmetric unit. A total of 1462 unique reflections were collected with CuKα radiation and 0:20 scans. Of these 1375 (94%) were judged observed [$|Fo| \geq 6\sigma(Fo)$] and used in further calculations. The structure was solved by direct methods and refined by full-matrix least-squares techniques to a final discrepancy index of 0.062 for the observed data.

The ^1H-NMR spectrum of **5** showed four downfield signals at δ 6.41, 7.42 and 7.46 which were assigned to the C-14, C-15, C-16 and C-17 protons, respectively, of the furanoid moiety. The C-12 methine proton of the six-membered lactone ring appeared as a doublet of doublet at δ 5.64. The ^{13}C-NMR spectrum ($CDCl_3$) of menispermacide (**5**) was assigned on the basis of DEPT and 2D HETCOR spectra. The *m/z* 359 in the mass spectrum represented a M^+-$SSCH_3$ ion. The other major peaks in the mass spectrum occurred at *m/z* 358 $C_{20}H_{22}O_6$), 365 ($C_{14}H_{17}O_5$), 219 ($C_{13}H_{15}O_3$), 95 (C_6H_7O) and 81 (C_5H_5O).

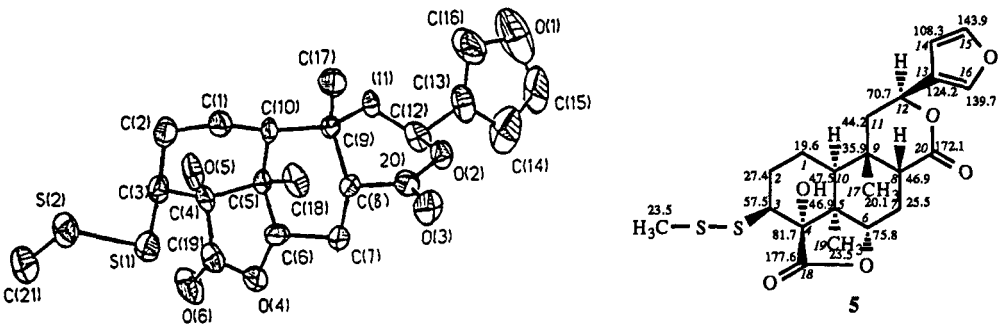


Figure 1: Structure of **5** is given on the right and computer generated perspective drawing of the final X-ray model is given on the left. No absolute configuration is implied.

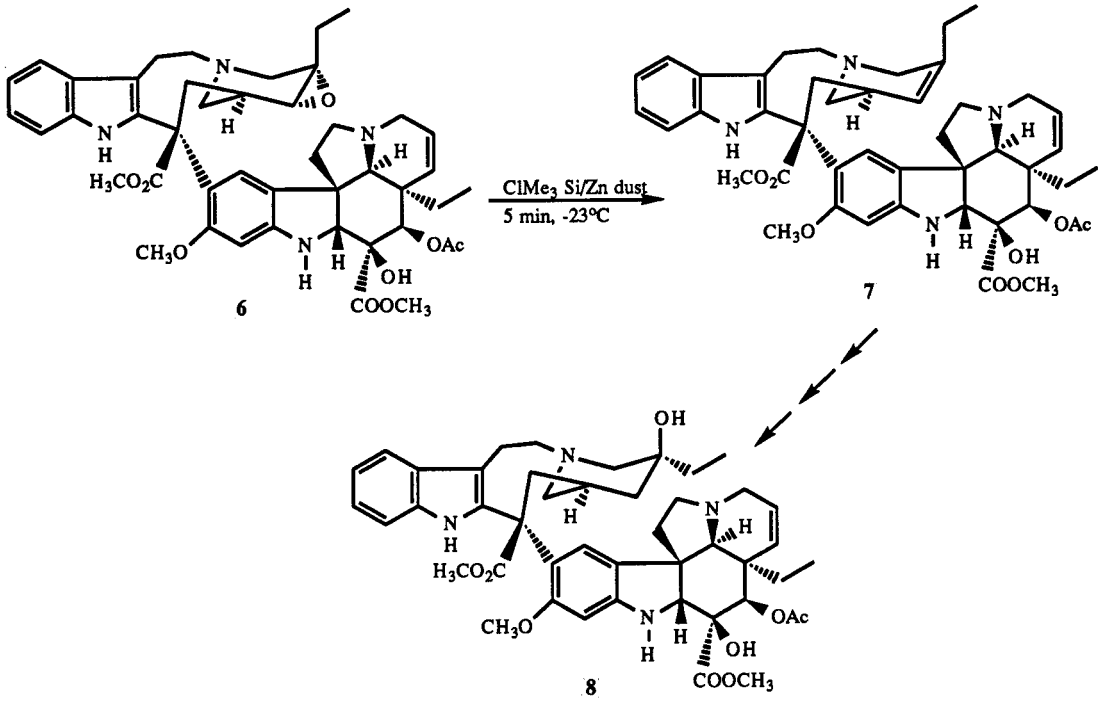
Biomimetic Syntheses of Vinblastine, Vincristine and their Analogue

The origin of the *tetracyclic* indole moiety of vinblastine had been the subject of much speculation during the 1960s and it was not at all obvious what the precursor to the top tetracyclic half of vinblastine was in nature. It was generally believed that tetracyclic "cleavamine" units having nine-membered *N*-containing rings were probable precursors to vinblastine and that such 16-hydroxycleavamine derivatives, which were perhaps too reactive to be isolated, could undergo facile combination with vindoline in the plant to afford vinblastine analogues. This view was challenged by us, since inspite of intensive investigations on the leaves of *Catharanthus roseus*, such 16-hydroxylated tetracyclic indole moieties with nine-membered nitrogen containing rings ("cleavamines") had not been isolated previously. This led us to propose a novel biosynthetic hypothesis in 1971 (11), which envisaged that vinblastine and vincristine may be derived from the *Iboga* alkaloid catharanthine (a *pentacyclic* compound) by attack of vindoline at C-16 of catharanthine. This attack could be concomittantly accompanied by cleavage of the C-16/C-21 bond of catharanthine to generate the "cleavamine" moiety. Subsequent hydration of the double bond could lead to vinblastine. We accordingly converted catharanthine into 16-carbomethoxycleavamine and then combined it with vindoline *via* the chloroindolenine to obatin the first synthetic analogue of vinblastine starting from catharanthine (11).

The work of our group (11) thus demonstrated for the first time that catharanthine (**6**), which is a major alkaloid in the leaves of *Catharanthus roseus*, could be utilized as the starting material for the synthesis of vinblastine analogues, and it marked a turning point in synthetic approaches to vinblastine and its analogues. Indeed all subsequent efforts by the Canadian (12) and French (13, 14) groups directed towards the synthesis of vinblastine, were carried out using catharanthine as the key precursor, and converting it into vinblastine, as first proposed and demonstrated by our group (11). This work opened the door to a host of semisynthetic biomimetic approaches to vinblastine analogues culminating in the first two syntheses of vinblastine itself in 1976 and 1978 by our group (15, 16). The first of these approaches (15) involved functionalization of catharanthine under modified Prevost conditions prior to coupling with vindoline, conditions under which catharanthine itself tends to decompose into a number of products. Since the reaction proceeded in variable yields which were difficult to reproduce, an improved approach was therefore developed by us involving functionalization of the tetracyclic moiety after coupling of catharanthine with vindoline (16) utilising the Potier modification of Polonovski reaction (14). An official patent was filed with the Government of Pakistan Patent Office (16) on 14th February 1978 (Government of Pakistan Patent No. 126852), *well over a year before an identical synthetic route to vinblastine was reported by the French group* (13). The ful text of our patent has been published (17).

Conversion of Leurosine to Anhydrovinblastine and Vinblastine

The alkaloid leurosine (**6**) is a major binary alkaloid present in the leaves of *C. roseus*, and it occurs in 10-20 fold higher yields than vinblastine. Various reactions involving its conversion to anhydrovinblastine (**7**) and then to vinblastine (**8**) have been explored by our group. A one step procedure for the quantitative conversion of leurosine (**6**) to anhydrovinblastine (**7**) using chlorotrimethylsilane/Zn dust has been developed by us (Scheme-**1**) (18). The reaction proceeds to completion within 5 minutes at -23°C. Since anhydrovinblastine (**7**) can then be converted to vinblastine by the procedure previously reported by us, this represents a new partial synthesis of vinblastine (**8**). A high yield conversion of leurosine (**6**) to anyhydrovinblastine was also accomplished by reaction of leurosine with phosphorous pentachloride in freshly distilled dry dichloromethane at room temperature, under an inert atmosphere of argon. The same reaction was also seen to occur on treatment of leurosine with atomized lithium or sodium metal in freshly distilled dry methylene chloride at room temperature (18).



Scheme-1

The CD *In Situ* Complexation Method as A Tool for Determination of Absolute Configuration of Cottonogenic Derivatives:

An easy and versatile method has been developed for the generation of enhanced Cotton effects (CEs) from optically active substance that are weakly absorbing in the accessible wavelength range. This method involves the *in situ* interactions of chiral careboxylic acids, α-amino acids, amino alcohols, ephedrine isomers and polynucleic acid with trinuclear metal complex: $[M_3O(O_2CCH_3)_6L3]^{n+}$, where M = Fe, Cr, Mn, Rh, Ru, etc., L = water or pyridine and n = 0 or 1. The following metal complexes were prepared:

(I) = $[Fe_3O(OOCCH_3)_6(H_2O)_3]$ = (FE 1); (II) = $[Fe_3O(OOCCH_3)_6(H_2O)]$. $5H_2O$ = (FE4)
(III) = $[Cr_3O(OOCCH_3)_6(H_2O)_3]$. $5H_2O$ = (CR1); (IV)$[Mn_3O(OOCCH_3)_6(Py)_3]$ ClO_4 = (MN1).

These derivatives with their conformational flexibility either reduced or totally restricted give rise to CEs. Semiemperically based helicity rules and newly established sector rules have been applied for correlation of the CEs with the absolute configuration (19).

Chiral Carboxylic Acids: Five optically active carboxulic acids were measured with CR1, where compounds **9** and (*S*)-(-)-3-phenyllactic acid (**10**) give identical CD spectra characterized by an intense negative CD around 600 nm and relatively minor positive CE around 450 nm. Compound **11** is the enatiomer of compound **9** and therefore shows the reverse CD spectra. Of the several CEs, the largest negative one which occurs at about 570 nm and which is common to compounds (**12**) and (*S*)-(-)-2-O-methyl-3-phenyllactic acid (**13**) may help in the determination of the absolute stereochemistry (Figure-2). The net CD remains negative for the chiral carboxylic acids with *S*-configuation and positive for carboxylic acids with *R*-configuration.

Amino Acids: The CD spectra of *in situ* complexes of 14 amino acids acting as ligands for the oxo-bridged metal carboxylates FE1, FE4, CR1 and MN1 has been measured. In general amino acids of the *R*-series yield a negative CD with complexes I, II, and IV. The reverse holds, of course, for the *S*-series.

Glycols: The CD of three open-chain glycols **13-15** were examined with MN1 complex, where one observed two distinct broad CEs between 600 and 500 and 500 and 400 nm which for **13** and **15** are associated with negative and positive signs, while for these are positive and negative, respectively (Figure-3). As mentioned, the glycols follow helicity rules and therefore one can correlate the diagonstic CEs with the torsion angle.

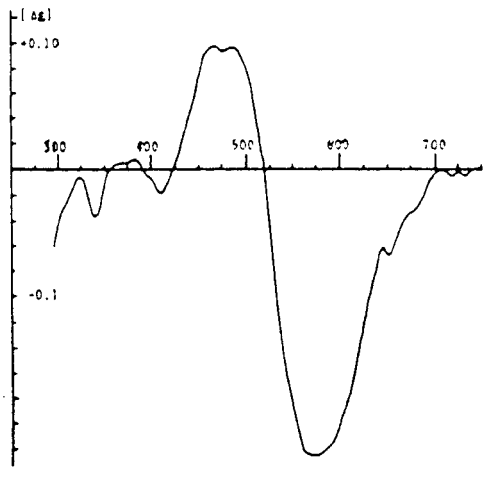


Figure-2: CD spectrum of (*S*)-(+)-mandelic acid in acetonitrile in TMP in the presence of CRI.

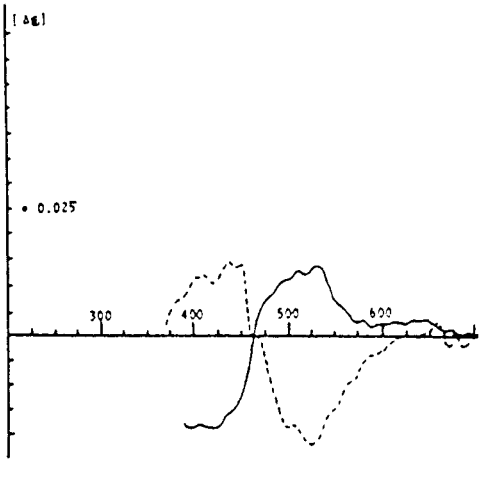
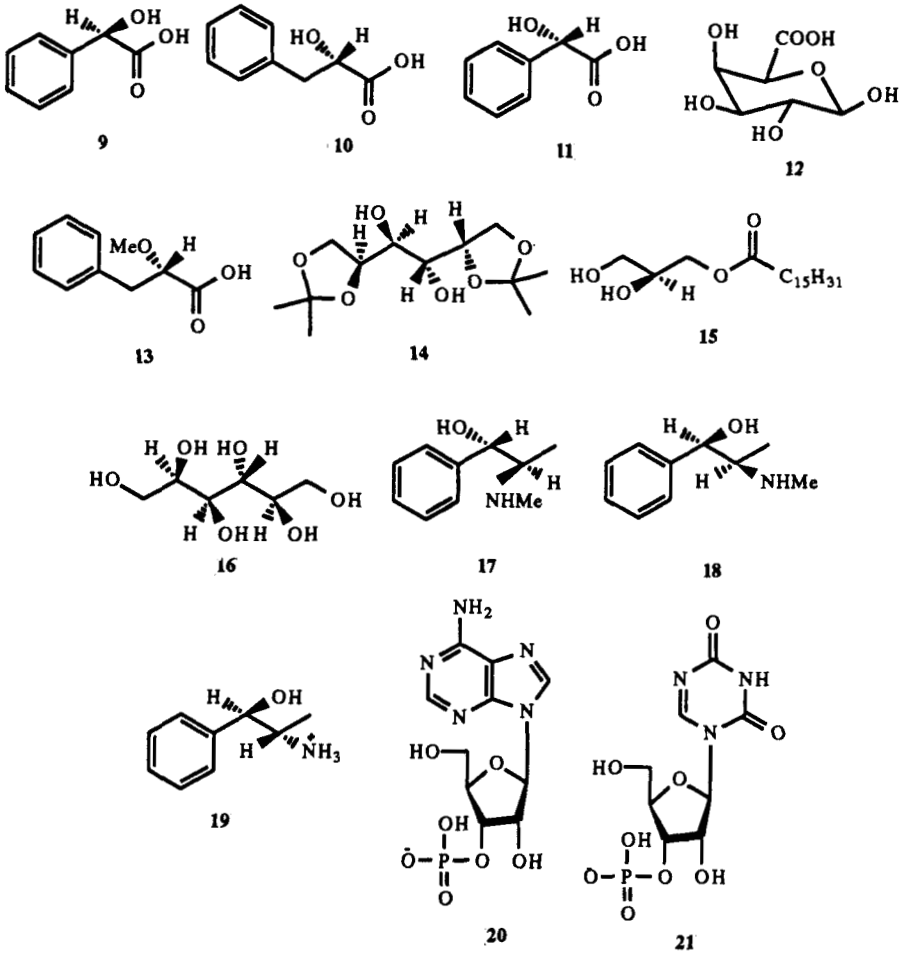


Figure-3: CD spectra of L-α-stearine (—) in acetonitrile and D-sorbitol (----) in ethanol in the presence of MNI.

Amino Alcohols: The results of CD measurements lead to the following conclusions: generally, open-chain amino alcohols of the L-series yield with I-IV the negative, longer wavelength CEs and the positive, shorter wavelength CEs, while the *D*-series give the positive longer wavelength CEs and negative, shorter wavelength CEs (19).

Ephedrine Isomers: Ephedrine isomers follow the semiemperically based helicity rules. In general, *erythro* compounds with 1*S*, 2*R* configurations such as **17** obey M-helicity, while those with 1*R*, 2*S* configurations such as **18** follow P-helicity. In the *threo* series, the compounds with 1*S*, 2*S* configuration such as **19** follow M-helicity, while those with 1*R*, 2*R* configurations obey P-helicity.

Polynucleic Acids: From the general consideration of the CD spectra of polynucleic acids we conclude that those aromatic bases with a ribose moeity containing at least two free OH groups generate Cotton effects. Enhanced CEs are however observed when the three hydroxyl groups are not replaced by the phosphate groups. Phosphorylation at position 3' as in **20** and **21** does not drastically change the CD spectrum. On the other hand, phosphorylation of the hydroxyl group at C-5' lead to the dramatic change in the CD spectra and in most cases no CD could be observed. Polynucleic acids may follow helicity rules which make use of torsion angle for the determination of the absolute configuration.



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