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Agrostophyllol and Isoagrostophyllol, Two Novel Diastereomeric 9,10-Dihydrophenanthropyran Derivatives from the Orchid *Agrostophyllum callosum*

P.L. Majumder*, S. Sen and S. Banerjee

Department of Chemistry, University College of Science
92, Acharya Prafulla Chandra Road, Calcutta 700 009, India

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Abstract - Agrostophyllol and isoagrostophyllol, two novel diastereomeric 9,10-dihydrophenanthropyran derivatives isolated from the orchid *Agrostophyllum callosum*, were shown to have the structures 2,7-dihydroxy-6-methoxy-9,10-dihydro-5H-phenanthro[4,5-bcd]pyran-5(ax)ol (**1l**) and 2,7-dihydroxy-6-methoxy-9,10-dihydro-5H-phenanthro[4,5-bcd]pyran-5(equat)ol (**1n**), respectively, from spectral and chemical evidence. **1l** and **1n** were shown to be the racemic mixtures of **1l'** and **1l"**, and **1n'** and **1n"**, respectively.

We reported earlier[1-12] the isolation of a fairly large number of compounds of diverse structural types from a series of Indian Orchidaceae plants. These compounds encompass a wide variety of stilbenoids, viz. bibenzyls,[1] phenanthrenes[2] and 9,10-dihydrophenanthrenes[3] and their dimers,[4] phenanthropyrans[5] and pyrones[5] and their 9,10-dihydro derivatives,[6] fluorenones,[7] besides several triterpenoids[8] and steroids[9] of biogenetic importance, a few flavonoids[10] and lignans[11] and a number of simple aromatic compounds.[12] As part of this general programme of research, we chemically investigated the orchid *Agrostophyllum callosum* which earlier afforded seven new stilbenoids, viz. callosinin[13a] (**1a**), callosumidin[13b] (**1b**), callosin[13a] (**2a**), callosumin[13b] (**2b**), agrostonin[13c] (**3a**), agrostonidin[13c] (**3b**) and callosuminin[13b] (**4**), besides 4-hydroxy-3,5-dimethoxybenzoic acid and the known stilbenoids imbricatin[6c,13c] (**1d**), flaccidin[6d,13a] (**1f**), oxoflaccidin[5b,13a] (**1h**), isooxoflaccidin[5c,13a] (**1i**), agrostophyllin[5a,13a] (**5a**), flaccidinin[5b,13a] (**5b**), orchinol[12,14a,14b] (**2c**) and 6-methoxycoelonin[13a,14c] (**2d**). Further chemical investigation of this orchid has now resulted in the isolation of yet two more new stilbenoids, designated agrostophyllol and isoagrostophyllol, which were shown to have the structures **1l** and **1n**, respectively, from the following spectral and chemical evidence.

Both agrostophyllol (**1l**) and isoagrostophyllol (**1n**) were shown to have the same molecular formula $C_{16}H_{14}O_5$ from elemental analysis and their mass spectrometrically derived molecular weight of 286. Both the compounds showed typical benzenoid UV absorptions [**1l** : λ_{max}^{EtOH} 223, 282 and 309 nm (log ε 3.88, 3.70 and 3.59); **1n** : λ_{max}^{EtOH} 220, 282 and 303 nm (log ε 4.39, 4.19 and 4.06)] resembling those of the 9,10-dihydrophenanthropyrans.[6] The phenolic nature of the compounds was indicated by their characteristic colour reactions [$FeCl_3$: violet; phosphomolybdic acid reagent : intense blue], alkali-induced bathochromic shifts of their UV maxima and their broad IR absorption band at 3380 cm^{-1}. The presence of two phenolic hydroxyl

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groups in each compound was confirmed by the formation of their respective diacetyl derivatives **1m** and **1o**, having the same molecular formula $C_{20}H_{18}O_7$ (M^+ 370), with Ac_2O and pyridine.

The ^1H NMR spectra of both **1l** and **1n** showed striking resemblance to those of imbricatin (**1d**) and flaccidin (**1f**) [Table 1], except that the signals for the oxymethylene protons of **1d** and **1f** [**1d** : δ 5.06; **1f** : δ 5.01] were replaced by one-proton signals at δ 6.57 and 6.92 in the spectra of **1l** and **1n**, respectively, which could be attributed to a benzylic lactol methine proton. This would suggest that while the two phenolic hydroxyl groups and the methoxyl functions in both **1l** and **1n** were distributed between C-2, C-3 and C-7* as in **1d** and **1f**, the oxymethylene bridge of the latter compounds were replaced by a lactol moiety in both **1l** and **1n**. The exact positions of the two phenolic hydroxyl groups at C-2 and C-7, and the methoxyl function at C-3 in both **1l** and **1n**, as in imbricatin (**1d**) rather than those in flaccidin (**1f**) having the two hydroxyls at C-3 and C-7 and the methoxyl at C-2, were ascertained by the fact that all the three aromatic protons of both **1l** and **1n** like those of **1d** showed the expected downfield shifts in the spectra of their respective diacetates **1m** and **1o**. The relative positions of the methoxyl and phenolic hydroxyl functions in **1l** and **1n** were also corroborated by the striking similarities of the chemical shifts and the splitting patterns of the aromatic protons of **1m** and **1o** with those of callosumidin diacetate (**1c**). The generation of a chiral centre at the lactol methine carbon of **1l**, **1m**, **1n** and **1o**, and the corresponding ethoxy-bearing carbon of **1c** was clearly evident by the fact that while the four benzylic protons of **1d**, **1e**, **1f** and **1g** resonated as four-proton singlets [**1d** : δ 2.70; **1e** : δ 2.87; **1f** : δ 2.86; **1g** : δ 2.86], the corresponding four benzylic protons of **1l**, **1m**, **1n**, **1o** and **1c** appeared as multiplets [**1l** : δ 2.84; **1m** : δ 2.87; **1n** : δ 2.84; **1o** : δ 2.77; **1c** : δ 2.81] due to nonequivalency of their protons at C-10. Based on the above spectral data both agrostophyllol and isoagrostophyllol were represented by the same gross structure **1j** which differs from imbricatin (**1d**) essentially by the replacement of one of the oxymethylene protons of **1d** by a hydroxyl group. It is interesting to note that the difference between the ^1H NMR spectra of agrostophyllol (**1l**) and isoagrostophyllol (**1n**) and their respective diacetates **1m** and **1o** lies essentially in the chemical shifts of their aromatic methoxyl protons which appeared at the normal region in the spectra of **1l** and **1m** similar to those of **1d**, **1e** and **1c** [**1l** : δ 3.85; **1m** : δ 3.84; **1d** : δ 3.70; **1e** : δ 3.77; **1c** : δ 3.79], while the corresponding protons in the spectra of **1n** and **1o** showed remarkable upfield shifts [**1n** : δ 3.14; **1o** : δ 3.01].

The gross structure **1j** for both agrostophyllol and isoagrostophyllol was also supported by the ^{13}C NMR spectral data of their more soluble diacetyl derivatives **1m** and **1o** (Table 2). The degree of protonation of the carbon atoms was determined by DEPT experiments and the carbon chemical shifts were assigned by comparison with the δ_c values of structurally similar compounds like **1e**, **1g** and **1c**. Thus, the δ_c values of all

*Although **1l**, **1n** and their derivatives were designated by systematic nomenclature, the phenanthrene numbering system is followed in this paper for convenience of ^1H and ^{13}C NMR spectral comparison.

Table 1. ^{1}H NMR spectral data of **1l, 1m, 1n, 1o, 1c, 1d, 1e, 1f** and **1g**

	Chemical shifts : δ ppm (Multiplicity; J in Hz								
Protons	**1l**$^{++}$	**1m**$^{+}$	**1n**$^{+++}$	**1o**$^{+}$	**1c**$^{+}$	**1d**$^{++}$	**1e**$^{+}$	**1f**$^{+}$	**1g**$^{+}$
H-1	6.79(s)	6.91(s)	6.73(s)	6.91(s)	6.89(s)	6.62(s)	6.84(s)	6.69(s)	6.66(s)
H-6	6.33(d) J=3	6.59 (ill-res. *m*-coupled d)	6.45(d) J=3	6.60 (ill-res. *m*-coupled d)	6.58 (ill-res. *m*-coupled d)	6.15(d) J=2.5	6.56 (br.s)	6.25(d) J=2.2	6.46 (br.s)
H-8	6.37(d) J=3	6.63 (ill-res. *m*-coupled d)	6.65(d) J=3	6.85 (ill-res. *m*-coupled d)	6.61 (ill-res. *m*-coupled d)	6.25(d) J=2.5	6.56 (br.s)	6.34(d) J=2.2	6.46 (br.s)
H_2-9 & H_2-10	2.84(m)	2.87(m)	2.84(m)	2.77(m)	2.81(m)	2.70(s)	2.87(s)	2.86(s)	2.86(s)
Ar-O*Me*	3.85 (s)	3.84 (s)	3.14 (s)	3.01 (s)	3.79 (s)	3.70 (s)	3.77 (s)	3.83 (s)	3.80 (s)
Ar-OH	6.37 & 6.44 (each 1H,br.s)	-	8.50 & 8.00 (each 1H,br.s)	-	-	8.20 (2H,br.s)	-	7.61 & 8.46 (each 1H,s)	-
Ar-OC*H*(R)-Ar	6.57(s) [R=OH]	6.58(s) [R=OH]	6.92(s) [R=OH]	6.83(s) [R=OH]	6.26(s) [R=OEt]	5.06 (s) [R=H]	5.22 (s) [R=H]	5.01 (s) [R=H]	5.01 (s) [R=H]
Ar-OCO*Me*	-	2.28 & 2.32 (each 3H,s)	-	2.20 & 2.27 (each 3H,s)	2.27 & 2.34 (each 3H,s)	-	2.27 & 2.33 (each 3H,s)	-	2.25 & 2.31 (each 3H,s)
OCH_2*Me*	-	-	-	-	3.83 (2H,m) 1.15 (3H,m)	-	-	-	-

$^{+}$ Spectra were run in $CDCl_3$; $^{++}$ Spectra were run in d_6-acetone; $^{+++}$ Spectrum was run in d_6-DMSO.

but the lactol methine carbon, C-5 and C-6 of **1m** and **1o** were virtually identical with those of the corresponding carbon atoms of **1e** indicating identical aromatic substitution in all the three compounds. This was also supported by the virtually identical δ_C values of the carbon atoms constituting the 9,10-dihydrophenanthrene parts of **1m**, **1o** and **1c** and also by the fact that while the δ_C values of C-7, C-8, C-8a, C-9, C-10 of **1m** and **1o** were almost the same as those of the corresponding carbon atoms of **1g**, there were substantial differences in the δ_C values of C-10a, C-4, C-4a, C-3, C-2 and C-1 of **1m** and **1o** from those of the corresponding carbon atoms of **1g** due to the interchange of the acetoxy and methoxy groups between C-2 and C-3. That one of the hydrogen atoms of the oxymethylene carbon of **1e** resonating at δ_C 63.1 was replaced by a hydroxyl group in both **1m** and **1o** was indicated by the appearance of the methine carbon signals at δ_C 88.7 and 87.7 in the spectra of **1m** and **1o**, respectively, which corresponded to their respective lactol methine carbons. The upfield shifts of C-5 of **1m** and **1o** by 1.7-1.9 ppm and the downfield shifts of their C-6 by 1.3-1.4 ppm compared to the corresponding carbon atoms of **1e** were also in agreement

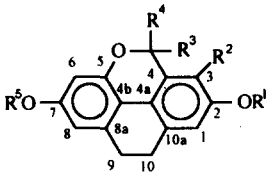
Table 2. ^{13}C NMR spectral data of **1m, 1o, 1c, 1e** and **1g**

C	δ_C (ppm)†				
	1m	**1o**	**1c**	**1e**	**1g**
1	123.4	123.4	123.1	121.5	111.1
2	143.3	142.7	142.7	142.2	150.2
3	145.7	146.6	146.1	145.2	137.5
4	121.9	121.4	121.9	122.2	122.3
4a	124.2	123.9	123.9	124.5	119.3
4b	115.5	115.5	114.9	116.3	116.8
5	151.2	151.0	151.1	152.9	152.5
6	109.2	109.1	108.8	107.8	108.2
7	149.9	149.4	150.3	150.6	150.6
8	114.6	114.9	114.4	114.1	114.3
8a	135.7	135.6	135.7	135.6	135.1
9	26.8[a]	26.6[b]	26.9[c]	26.4[d]	27.5[e]
10	27.4[a]	27.3[b]	27.4[c]	27.1[d]	27.6[e]
10a	129.5	129.2	129.4	128.9	131.6
Ar-O<u>C</u>HR-Ar	88.7 [R=OH (ax)]	87.7 [R=OH (equat)]	93.9 [R=OEt (ax)]	63.1 (R=H)	62.4 (R=H)
Ar-O<u>C</u>H_3	61.6	61.3	61.7	61.0	56.1
Ar-O<u>C</u>O<u>C</u>H_3	169.6	169.1	168.9	168.9	169.4
	168.7	168.7	168.9	168.7	168.6
	20.9	21.0	20.9	20.6	21.1
	20.6	20.6	20.6	20.3	20.3
-O<u>C</u>H_2<u>C</u>H_3	-	-	64.0, 15.0	-	-

†Spectra were run in $CDCl_3$ and the chemical shifts were measured with $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.
[a–e]Values are interchangeable within the same column.

with the replacement of the oxymethylene bridge of **1e** by a lactol moiety in both **1m** and **1o** and hence in **1l** and **1n**. The ethoxy bearing methine carbon of **1c**, as expected, appeared at a much lower field (δ_C 93.9) than the corresponding lactol methine carbons of **1m** and **1o**. Agrostophyllol diacetate and isoagrostophyllol diacetate were thus represented by the same gross structure **1k** which, in turn, further affirmed the gross structure **1j** for the parent compounds. The structures **1j** and **1k** were also supported by the appearance of the intense peaks at m/z 269 and 353 corresponding to the highly stabilized ion-fragments **a** and **b** in the mass spectra of the compounds and their diacetyl derivatives, respectively, formed by the expulsion of OH from the benzylic lactol methine carbon of the respective molecular ions.

The gross structure **1j** for both the compounds was finally confirmed by their reduction with $NaBH_4$

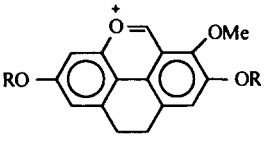


1a: R^1=R^5=Me, R^2=OMe, R^3=R^4=H
1b: R^1=R^3=R^5=H, R^2=OMe, R^4=OEt(ax)
1c: R^1=R^5=Ac, R^3=H, R^2=OMe, R^4=OEt(ax)
1d: R^1=R^3=R^4=R^5=H, R^2=OMe
1e: R^1=R^5=Ac, R^2=OMe, R^3=R^4=H
1f: R^1=Me, R^2=OH, R^3=R^4=R^5=H
1g: R^1=Me, R^2=OAc, R^3=R^4=H, R^5=Ac
1h: R^1=Me, R^2=OH, R^5=H, R^3,R^4=O
1i: R^1=R^5=H, R^2=OMe, R^3,R^4=O

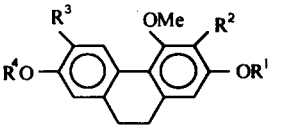
1j: R^1=R^5=H, R^2=OMe, R^3,R^4= 

1k: R^1=R^5=Ac, R^2=OMe, R^3,R^4= 

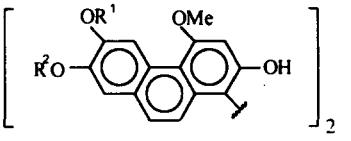
1l: R^1=R^3=R^5=H, R^2=OMe, R^4=OH (ax)
1m: R^1=R^5=Ac, R^2=OMe, R^3=H, R^4=OH (ax)
1n: R^1=R^4=R^5=H, R^2=OMe, R^3=OH (equat.)
1o: R^1=R^5=Ac, R^2=OMe, R^4=H, R^3=OH (equat.)
1u: R^1=R^3=R^5=H, R^2=OMe, R^4=CH_2COCH_3 (ax)
1v: R^1=R^3=R^5=H, R^2=OMe, R^4=OMe (ax)



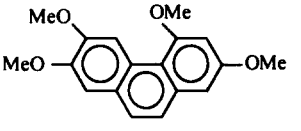
a: R = H (m/z 269)
b: R = Ac (m/z 353)



2a: R^1=R^2=H, R^3=OH, R^4=Me
2b: R^1=R^4=Me, R^2=H, R^3=OMe
2c: R^1=Me, R^2=R^3=R^4=H
2d: R^1=R^2=R^4=H, R^3=OMe



3a: R^1=Me, R^2=H
3b: R^1=H, R^2=Me



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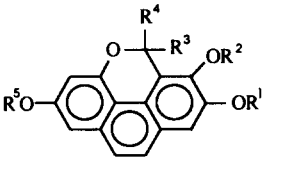
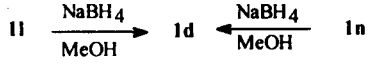


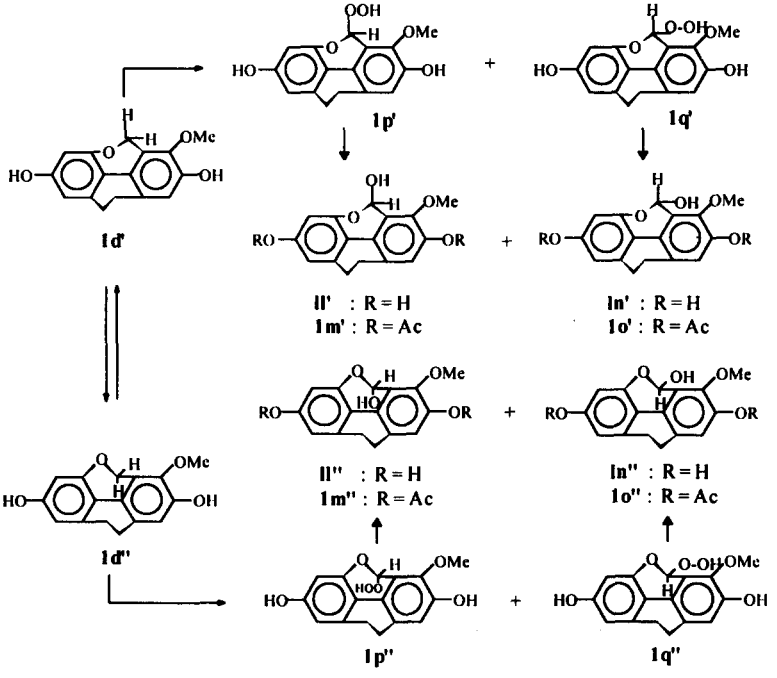
5a: R^1=R^3=R^4=H, R^2=R^5=Me
5b: R^1=Me, R^2=R^5=H, R^3,R^4=O



in MeOH to imbricatin (**1d**) identified by direct comparison with an authentic sample.[6c]

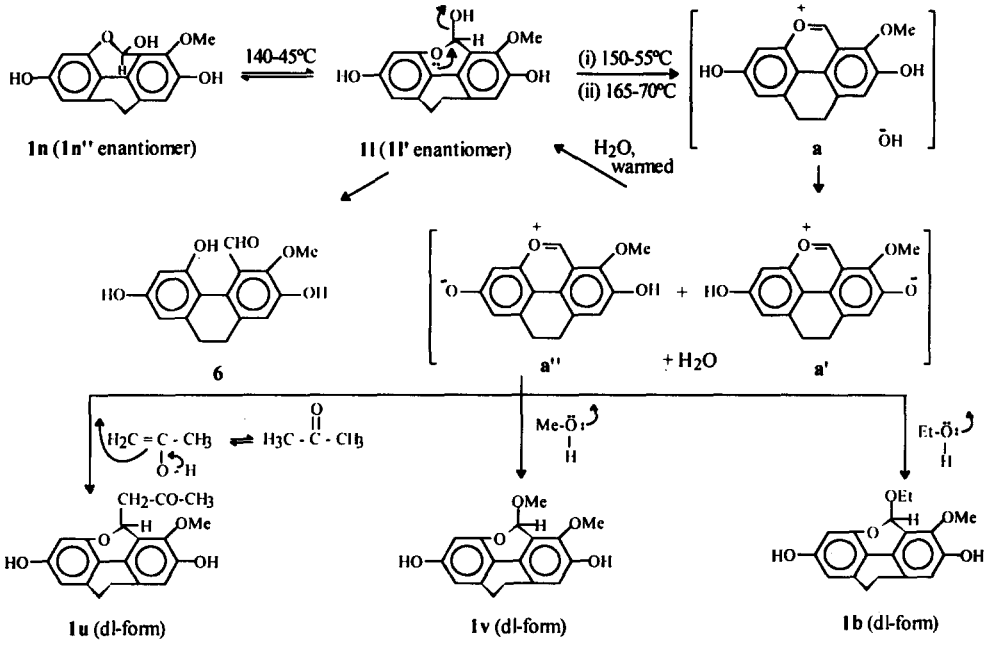
Construction of Dreiding models for **1j** shows that it may exist as four stereoisomers **1l'**, **1l''**, **1n'** and **1n''** comprising a pair of diastereomers, each of which being a racemic mixture as evident from the fact that both agrostophyllol and isoagrostophyllol were found to be optically inactive. Agrostophyllol and isoagrostophyllol may, therefore, be represented by the alternative dl-pairs **1l'** and **1l''**, and **1n'** and **1n''** and their respective diacetates by the dl-pairs **1m'** and **1m''**, and **1o'** and **1o''**. A decision between the two alternative possibilities for each diastereomer was made by the chemical shifts of the aromatic methoxyl protons of the compounds and their respective diacetyl derivatives. In **1l'** and **1l''** and the corresponding

diacetates **1m'** and **1m"**, the aromatic methoxyl group at C-3 being far off from the lactol OH would be expected to resonate at the normal region. On the other hand, in **1n'** and **1n"** (and hence in the corresponding diacetates **1o'** and **1o"**), the aromatic methoxyl group at C-3 being flanked by the much closer lactol OH and the hydroxyl group at C-2 (acetoxy group at C-2 in **1o'** and **1o"**) are thus forced out-of-plane of the aromatic ring. Consequently, the protons of the aromatic methoxyl group in **1n'** and **1n"** as well as in **1o'** and **1o"** would be expected to resonate at an upfield position. Now, the appearance of the aromatic methoxyl protons in the ^{1}H NMR spectra of agrostophyllol (**1l**) and its diacetate **1m** at the normal positions [**1l** : δ 3.85; **1m** : δ 3.84] suggests that they are the dl-pairs of **1l'** and **1l"**, and **1m'** and **1m"**, respectively. On the other hand, the upfield resonances of the corresponding aromatic methoxyl protons of isoagrostophyllol (**1n**) and its diacetate **1o** [**1n** : δ 3.14; **1o** : δ 3.01] implies that they must be the dl-pairs of **1n'** and **1n"**, and **1o'** and **1o"**, respectively. Looking at the conformational structures **1l'**, **1l"**, **1n'** and **1n"** of the four possible stereoisomers constructed from the gross structure **1j**, it is apparent that **1l'** and **1l"** are the flipped forms of **1n"** and **1n'**, respectively. The same relation exists between **1m'** with **1o"** and **1m"** with **1o'**. But while the conformers **1d'** and **1d"** of imbricatin (**1d**) having no lactol OH are readily interconvertible at room temperature, no equilibration between agrostophyllol (**1l**) and isoagrostophyllol (**1n**) was observed on separately heating the solution of the compounds in boiling EtOAc for 8 h and even on heating the solution of **1n** in EtOAc or $CHCl_3$-Me_2CO (1:1) in a teflon screw-capped glass tube at 130-35°C in an oil bath for 4 h. But when the



above solutions of **1n** in the aforesaid sealed-tubes were separately heated to 140-45°C for 1 h, the resultant solutions showed the presence of only **1n** and **1l** in the ratio of *ca* 9:1 indicating that equilibration of **1n** with **1l** started slowly only at 140-45°C. The ratio of **1n** and **1l** became *ca* 7:3, when the above solutions were separately heated at the same temperature for a further period of 5 h and remained the same on heating the above sloutions for a period of another 3 h indicating the attainment of equilibrium between the two isomers. In the above and subsequent thermal studies, the conversion of **1n** to **1l** was monitored by TLC [The two isomers have distinctly different R_f values in petrol-EtOAc (3:2) solvent system.] and the ratios of **1n** and **1l** in the mixtures examined at different time intervals were determined from the integrated intensities of the readily identifiable methoxyl proton signals of the two isomers in the ^{1}H NMR spectra of the residues obtained at the end of each time interval. When the sealed-tubes containing the above equilibrium mixture of **1n** and **1l** in EtOAc and $CHCl_3$-Me_2CO were separately heated to 150-55°C for 2 h, TLC of the resultant solutions showed, in the first case (EtOAc), the presence of a highly polar compound [which did not move from the base line even with EtOAc as the solvent system as indicated by the iodine-stained spot in the chromatogram] in addition to **1l** and **1n** in the ratio of *ca* 6:1:3, while that in the second case ($CHCl_3$-Me_2CO) indicated the presence of a nonpolar compound, **1l** and **1n** almost in the same ratio. When the above solutions were separately heated for a further period of 2 h at 165-70°C, the resultant solution in EtOAc was found to contain the polar compound, **1l** and **1n** in the ratio of *ca* 17:2:1 and that in $CHCl_3$-Me_2CO, the nonpolar compound, **1l** and **1n** were also found almost in the same ratio. The residues obtained after heating **1n** in EtOAc separately for 2 h (at 150-55°C) and for 4 h (2 h at 150-55°C and 2 h at 165-70°C), when warmed with little water, showed the total absence of the polar compound in each case and the resultant products found to contain only **1l** and **1n** in the ratio *ca* 7:3 and 19:1, respectively, as evident from their TLC and ^{1}H NMR spectra. The ^{1}H NMR spectra of the above residues before treatment with water showed, besides the usual signals for **1l** and **1n**, some additional signals, one of which appeared at δ 8.02 (s). The signal at δ 8.02 may be attributed to a proton of the oxonium moiety Ar-O^+=CH-Ar of a dibenzopyrylium salt. The residue obtained after heating **1n** in EtOAc at 165-70°C as stated above, when warmed separately with dry MeOH and EtOH, afforded mostly **1v** and callosumidin[13b] (**1b**), respectively. The structure of **1v** was established mainly from its ^{1}H NMR spectrum showing, besides the usual signals, a signal at δ 3.52 for an aliphatic methoxyl group. The conversions of the polar compound to **1b** and **1v** on treatment with EtOH and MeOH were also achieved during TLC of the above residue run in 5% ethanolic and methanolic $CHCl_3$, respectively. The same conversions were also made by preparative TLC of the above residue and taking out the silica gel from the base line area of the chromatograms, followed by warming the above silica gel with EtOH and MeOH. Although the polar compound could not be isolated, its mode of formation, the ^{1}H NMR spectrum of the compound in mixture and its reaction products with water, MeOH and EtOH provided strong evidence for the

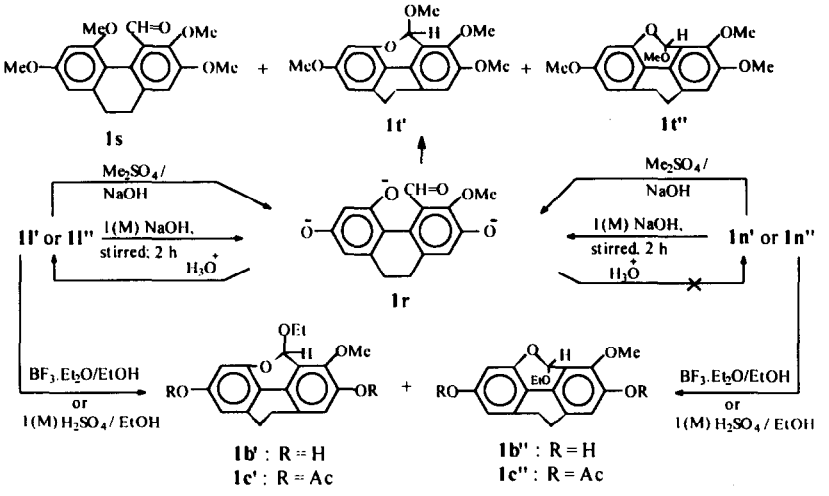
presence of a dibenzopyrylium moiety in the compound. It seems likely that the compound may have the structure **a'** or **a''** or a mixture of both, which are formed through the intermediacy of **a** generated from **1l** by the elimination of the stereochemically favourable axial lactol OH having an adjacent donor oxygen atom (Scheme 1). The ⁻OH thus eliminated, during the formation of **a**, presumably reacts with its phenolic hydroxyl



Scheme 1 : Conversions of **1n** to **1l** and **1l** to **a**, **a'**, **a''**, **1u**, **1v** and **1b** and regeneration of **1l** from **a'** and **a''**.

groups to give **a'** or **a''** or both. Water formed during the formation of **a'** or **a''** is mostly removed from the reaction phase at the high temperature of the reactions (150-70°C) and mostly condenses on the upper walls of the sealed tube. Addition of more water to the reaction mixture regenerated the thermodynamically more stable isomer **1l** by nucleophilic attack on the pyrylium moiety of **a'** or **a''** followed by hydrolysis of the phenolate ion. The possibility of the polar compound having a phenolic aldehyde structure **6** seems unlikely in view of the facts that (i) a compound of the structure **6** should not have such high polarity; (ii) the proton resonance at δ 8.02 of the compound is too upfield for an aldehyde proton; (iii) it is difficult to rationalise the formation of **1b** and **1v** by the reaction of **6** with EtOH and MeOH, respectively, under the reaction conditions stated above. The nonpolar compound obtained with $CHCl_3$-Me_2CO was shown to have the structure **1u** mainly from its ^1H NMR spectral data and was assumed to be formed by the nucleophilic attack by the enol form of acetone on **a'** or **a''**. The formation of **a**, **a'**, **a''** and **1u** (Scheme 1) thus facilitates the shift of the equilibrium from **1n** to **1l**. Such high energy required for the conversion of **1n** to **1l** may be rationalized by the fact that at the transition state of flipping of **1n** and **1l** the lactol OH and the OMe group at C-3 so heavily

impinge on each other appearing well within their van der Waals radii that flipping of **1l** and **1n** involves an unusually high activation energy. However, conversion of **1n** to **1l** was readily achieved chemically by opening of the lactol ring (Scheme 2). Thus, when agrostophyllol and isoagrostophyllol were separately



Scheme 2: Conversion of **1n** to **1l** and the formation of **1b**, **1s** and **1t** from both **1n** and **1l**.

treated with 1(M) aq. NaOH solution, both gave the same intermediate aldehydic phenoxide ion **1r** by opening of the lactol ring. Recyclization of **1r** afforded the thermodynamically more stable isomer **1l** (the dl form of **1l'** and **1l"**). In this reaction, only isoagrostophyllol is converted to agrostophyllol, the latter apparently remaining unchanged. Similarly, both agrostophyllol and isoagrostophyllol, when treated separately with Me_2SO_4/NaOH gave a mixture of the aldehyde **1s** and the racemic mixture of the thermodynamically more stable lactol methyl ether derivative **1t** (dl form of **1t'** and **1t"**) having ax-OMe, which were presumably formed by the same intermediate **1r**. The structures of **1s** and **1t** were established from their ^1H NMR spectral data. Again, treatment of agrostophyllol and isoagrostophyllol separately with $BF_3.Et_2O$/EtOH or 1(M) aq. H_2SO_4/EtOH gave only the thermodynamically more stable axial lactol ethyl ether derivative callosumidin[13b] (**1b**) [the dl form of **1b'** and **1b"**]. The formation of **1b** from **1l** or **1n** in the above reactions may be assumed to involve the intermediacy of the oxonium ion **a** generated by the removal of the lactol OH followed by nucleophilic attack by EtOH. Acetylation of **1b** formed in the above reactions afforded callosumidin diacetate **1c**[13b] (the dl forms of **1c'** and **1c"**).

Agrostophyllol (**1l**) and isoagrostophyllol (**1n**) may be assumed to be formed through the intermediacy of the four possible hydroperoxy derivatives **1p'**, **1q'**, **1p"** and **1q"** obtained from the readily interconvertible conformers **1d'** and **1d"** of imbricatin (**1d**) and represent two novel examples of otherwise conformational isomers becoming highly stable diastereomers due to unusual steric hindrance to conformational flipping.

EXPERIMENTAL

M.P.s. are uncorrected. UV spectra were measured in aldehyde-free EtOH and IR spectra were run in KBr discs. ^{1}H NMR spectra were recorded in Bruker 300 MHz supercon instrument using TMS as the internal standard and $CDCl_3$ as the solvent, unless otherwise stated. ^{13}C NMR were run at 75 MHz in the same instrument using the same internal standard. Chemical shifts were measured in δ ppm. Mass spectra were recorded at 70 eV using direct inlet system and the figures in the first bracket attached to m/z values represent relative intensities of peaks. Silica gel (100-200 mesh) was used for column chromatography (CC), silica gel (230-400 mesh) for Medium Pressure Liquid Chromatography (MPLC) and silica gel G for TLC. All analytical samples were routinely dried over P_2O_5 *in vacuo* for 24 h and were tested for purity by TLC and MS. Dry Na_2SO_4 was used for drying organic solvents and petrol used had b.p. 60-80°C.

*Isolation of agrostophyllol (**1l**) and isoagrostophyllol (**1n**), **1a**, **1b**, **1d**, **1f**, **1h**, **1i**, **2a**, **2b**, **2c**, **2d**, **3a**, **3b**, **4**, **5a** and **5b** from Agrostophyllum callosum.* Air-dried whole plant of *A. callosum* (5 kg) was soaked in 10 L of MeOH for 3 weeks. The methanolic extract was drained out and concentrated under reduced pressure to *ca* 100 ml, diluted with water (500 ml) and the liberated solids were extracted with Et_2O. The Et_2O layer was extracted with 2M aq. NaOH solution. The aq. alkaline solution was acidified with concentrated HCl in the cold and the liberated solids were extracted with Et_2O, dried and the solvent removed. The residue was chromatographed. Compounds **1a, 1b, 1d, 1f, 1h, 1i, 2a, 2b, 2c, 2d, 3a, 3b, 4, 5a, 5b** and 4-hydroxy-3,5-dimethoxybenzoic acid were isolated following the methods described earlier.[13] The petrol-EtOAc (5:1) eluate afforded a mixture of **1l** and **1n**. The mixture on repeated MPLC finally afforded pure **1l** (0.5 g, 0.01%) as a pale yellow solid, m.p. 190°C (recrystallized from petrol-EtOAc) and **1n** (0.4 g, 0.008%) as a white solid, m.p. >300°C (recrystallized from petrol-EtOAc). **1l** (Found : C, 67.01; H, 4.83. $C_{16}H_{14}O_5$ requires : C, 67.11; H, 4.93%). $[\alpha]_D^{25}$ 0 (c 1.1, MeOH); λ_{max} nm : 223, 282 and 309 (log ε 3.88, 3.70 and 3.59); $\lambda_{max}^{EtOH-0.1M\ NaOH}$ nm : 224, 298 and 318 (log ε 3.82, 3.77 and 3.74); IR ν_{max} (cm^{-1}) : 3380 (broad band; OH), 1610, 1450, 1350, 1300, 1000, 920, 850 (aromatic nucleus); MS : EI m/z 286 (M^+, 1), 270 (57), 269 (24), 255 (20), 237 (6), 225 (7), 152 (5), 139 (9) and 115 (5). **1l** was acetylated with Ac_2O and pyridine in the usual manner to give **1m** as a white solid, m.p. 140°C (recrystallized from petrol-EtOAc). (Found : C, 64.76; H, 4.84. $C_{20}H_{18}O_7$ requires : C, 64.84; H, 4.90%). $[\alpha]_D^{25}$ 0 (c 0.95, MeOH); λ_{max} nm : 219, 276, 299 and 310 (log ε 4.47, 4.10, 4.05 and 4.03); IR ν_{max} (cm^{-1}) : 3400 (OH), 1225 and 1760 (OAc), 1600, 1490, 1380, 1210, 1150, 1030, 910 and 750 (aromatic nucleus); MS : EI m/z 370 [M$^+$, 16], 354 (18), 353 (25), 328 (30), 312 (35), 286 (47), 284 (22), 270 (100), 268 (47), 254 (37), 253 (20), 237 (15), 225 (19), 197 (14), 165 (15), 152 (18), 139 (14), 115 (13), 86 (27), 84 (45), 49 (85) and 43 (44). **1n** (Found : C, 67.06; H, 4.81. $C_{16}H_{14}O_5$ requires : C, 67.11; H, 4.93%). $[\alpha]_D^{25}$ 0 (c 1.2, MeOH); λ_{max} nm : 220, 282 and 303 (log ε 4.39, 4.19 and 4.06); $\lambda_{max}^{EtOH-0.1M\ NaOH}$ nm : 218, 296 and 313 (log ε 4.35, 4.23 and 4.19) : IR ν_{max} (cm^{-1}) : 3380 (broad band, OH), 1625, 1450, 1300, 1150, 1000 and 930 (aromatic nucleus). **1n** was acetylated with Ac_2O and pyridine in the usual manner to give **1o** as a white solid, m.p. 215°C (recrystallized from petrol-EtOAc). (Found : C, 64.77; H, 4.81. $C_{20}H_{18}O_7$ requires; C, 64.84; H, 4.90%). $[\alpha]_D^{25}$ 0 (c 0.89, MeOH); λ_{max} nm : 218, 273 and 308 (log ε 3.84, 3.52 and 3.43); IR ν_{max} (cm^{-1}) : 3440 (OH), 1220 and 1770 (OAc), 1605, 1490, 1450, 1370, 1210, 1140, 1005 and 920 (aromatic nucleus); MS : EI m/z 370 [M$^+$, 6], 354 (21), 353 (100), 328 (6), 324 (9), 312 (11), 311 (24), 286 (8), 282 (15), 270 (24), 269 (21), 268 (16), 254 (22), 241 (15), 240 (10), 225 (9), 198 (5) and 43 (38).

*Reduction of **1l** and **1n** with $NaBH_4$* . To solutions of 0.05 g (0.175 mmol) of each of **1l** and **1n** in 10 ml of MeOH were added separately 0.02 g (0.529 mmol) of $NaBH_4$ with stirring. The mixtures were heated under reflux on boiling water bath for 1 h. MeOH was removed under reduced pressure and the residues were acidified with 2 M HCl in the cold and extracted separately with Et_2O, washed with water, dried and the solvent removed to give **1d**,[6c] crystallized from petrol-EtOAc, m.p. 144°C (lit.[6c] m.p. 145°C) in both the cases (0.046 g, 98% from **1l** and 0.045 g, 95% from **1n**), identified by direct comparison (m.m.p. and superimposable IR and ^{1}H NMR spectra) with an authentic sample.

*Thermal conversion of **1n** to **1l** and **a'/a"** and the reaction of **a'/a"** with H_2O, MeOH and EtOH.* Four sets of solutions of **1n** (each 0.03 g, 0.105 mmol) at 140-45°C in EtOAc (each 5 ml) were separately heated in sealed

tubes, one at 150-55°C for 1 h, the second at the same temperature for a further period of 5 h, the third at 150-55°C for 2 h (after heating for 6 h at 140-45°C) and the fourth at 165-70°C for 2 h (after heating first at 140-45°C for 6 h and then at 150-55°C for 2 h) and the residues A, B, C and D obtained after evaporation of solvent in the four sets of experiments, respectively, were examined by TLC and ^{1}H NMR (in d_6-acetone). The ratios of the compounds in the residues A, B, C and D were obtained from the integrated intensities of the signals for the protons of their methoxyl groups at C-3. The ^{1}H NMR spectra of the residues C and D (in d_6-acetone) showed, besides the usual signals for **1l** and **1n**, additional signals for **a'/a"** : δ 2.97 (4H, s; H_2-9, H_2-10), 3.85 (3H, s; Ar-OMe), 6.79 (1H, ill-resolved *m*-coupled doublet; H-8), 6.81 (1H, ill-resolved *m*-coupled doublet; H-6), 6.98 (1H, s; H-1), 7.34 and 7.60 (each br. signal; Ar-OH) and 8.02 (1H, s; Ar-O$^+$=CH-Ar).

Residue C and 1/3 of the residue D were separately treated with water (5 drops) and the resulting mixtures warmed and shaken for 5 min, extracted with EtOAc, dried and the solvent removed. The ^{1}H NMR spectra of the products from the residues C and D (in d_6-acetone) showed the presence of **1l** and **1n** in the ratio of *ca* 7:3 and 19:1, respectively. The remaining part of the residue D was divided into two equal parts, which were separately treated with dry MeOH and EtOH (each 1 ml) and warmed on water bath with shaking for 5 min. Excess MeOH and EtOH were removed and the residues were separately extracted with EtOAc, dried and the solvent removed. The residues were separately chromatographed. The petrol-EtOAc (2:1) eluate in the chromatography of the products obtained with MeOH gave, besides traces of **1l** and **1n**, **1v** (0.009 g, 80%), as white amorph. solid. **1v** ^{1}H NMR : δ 2.94 (4H, m; H_2-9, H_2-10), 3.52 (3H, s; Ar-O-CH (OMe)-Ar), 3.84 (3H, s; Ar-OMe), 6.21 (1H, s; Ar-OCH (OMe)Ar), 6.45 (1H, ill-resolved *m*-coupled doublet; H-6), 6.50 (1H, ill-resolved *m*-coupled doublet; H-8), 6.80 (1H, s; H-1), 7.39 and 8.0 (each 1H, s; Ar-OH). The chromatography of products obtained with EtOH gave in the petrol-EtOAc (2:1) eluate **1b**[13c] (0.0095 g, 81%), besides traces of **1l** and **1n**.

0.03 g of the residue D was subjected to preparative TLC on silica gel G using EtOAc as the solvent system. The silica gel from the lower part of the chromatograms were collected and were divided into two equal parts and separately heated with dry MeOH and EtOH for 5 min, filtered and the filtrates on removal of excess MeOH and EtOH gave **1v** (0.013 g, 77%) and **1b** (0.0135 g, 80%).

*Formation of **1u** during the thermal treatment of **1n**.* A solution of **1n** (0.01 g, 0.035 mmol) in $CHCl_3$-Me_2CO (1:1) (2 ml) in a sealed tube was first heated at 140-45°C for 1 h and then for 2 h at 150-55°C and finally at 165-70°C for a further period of 2 h. The residue after removal of the solvent was chromatographed. The petrol-EtOAc (5:1) eluate afforded **1u** (0.008 g, 70%). ^{1}H NMR of **1u** : δ 2.16 (3H, s; -CH_2CO*Me*), 2.44 (2H, AB q with fine splitting; -C*H*$_2$-COMe), 2.82 (4H, m; H_2-9 and H_2-10), 3.86 (3H, s; ArO*Me*), 5.40 and 5.50 (each br.s; ArOH), 5.99 (1H, dd, J_1=10.4 Hz and J_2=2.7 Hz; -O-C*H*-CH_2COMe), 6.27 and 6.33 (each 1H, d, J=2.2 Hz; H-6 and H-8) and 6.74 (1H, s; H-1). Further elution of the column with petrol-EtOAc (3:1) gave a mixture of **1l** and **1n**, which on repeated chromatography gave **1l** (0.001 g, 10%) and traces of **1n**.

*Reaction of **1l** and **1n** with 1M NaOH solution.* A solution of 0.05 g (0.175 mmol) of each of **1l** and **1n** in 5 ml 1 M aq. NaOH solution was separately stirred for 2 h and then acidified with conc. HCl in the cold. The residues were extracted separately with Et_2O, washed with water, dried and the solvent removed. The residue obtained from the reaction of **1n** was found to be identical with **1l** (0.049g, 98%), while that obtained from the reaction of **1l** was found to be unchanged **1l**.

*Reaction of **1l** and **1n** with Me_2SO_4 and NaOH.* 0.05 g (0.175 mmol) of each of **1l** and **1n** was separately treated with 15 ml 10% NaOH solution. The solutions were cooled to 10°C in ice-bath. 2 ml (21.1 mmol) of Me_2SO_4 were added dropwise with stirring to each of these solutions. The mixtures were then heated on a boiling water bath for 2 h with stirring. The reaction mixtures were acidified with dil. H_2SO_4 and extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residues were separately chromatographed to give **1s** (0.015 g, 26%) as pale yellow semi-solid mass and **1t** [dl pair of **1t'** and **1t"**] (0.03 g, 52%) as white amorp. solid in each case. **1s** (Found : C, 69.45; H, 5.99. $C_{19}H_{20}O_5$ requires : C, 69.48; H, 6.14%). ^{1}H NMR : δ 9.04 (1H, s; -CHO), 6.84 (1H, s; H-1), 6.34 (1H, d; J=2.4 Hz; H-8), 6.29 (1H, d; J=2.4 Hz; H-6), 3.88, 3.80, 3.79 and 3.77 (each 3H, s; 4xArOMe) and 2.75 (4H, m; H_2-9 and H_2-10). **1t** (Found : C, 69.47; H, 6.01. $C_{19}H_{20}O_5$ requires : C, 69.48; H, 6.14%). ^{1}H NMR : δ 6.75 (1H, s; H-1), 6.45 (1H, d; J=2.4 Hz; H-8), 6.39 (1H,

d; J=2.4 Hz; H-6), 6.15 (1H, s; Ar-O-C*H*(OMe)-Ar), 3.82, 3.81 and 3.75 (each 3H, s; 3xArO*Me*), 3.52 (3H, s; Ar-O-CH(O*Me*)-Ar) and 2.82 (4H, m; H_2-9 and H_2-10).

Reaction of **1l** *and* **1n** *with EtOH in presence of* $BF_3.Et_2O$ *and 1M aq.* H_2SO_4. 0.02 g (0.07 mmol) of each of **1l** and **1n** were separately treated with 1 ml EtOH in presence of 4 drops of BF_3. Et_2O. The mixtures were stirred at room temperature for 1 h. EtOH was removed under reduced pressure and the residue was treated with H_2O, extracted with Et_2O, dried and the solvent removed. The residues were separately chromatographed to give **1b** (0.018 g, 82% from **1l** and 0.015 g, 68% from **1n**). Again, 0.02 g (0.07 mmol) of each of **1l** and **1n** in 2 ml EtOH were separately treated with 5 drops of 1 M aq. H_2SO_4. The mixtures were stirred at room temperature for 2 h. EtOH was removed under reduced pressure and the residue in each case was extracted with Et_2O, dried and the solvent removed. The residues were separately chromatographed to give **1b** (0.016 g, 73% from **1l** and 0.014 g, 64% from **1n**).

Chromatography of the neutral part of *A. callosum* afforded **1a** (0.2 g, 0.004%), m.p. 101°C, **2b** (0.02 g, 0.0004%) and **4** (0.2 g, 0.004%).

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