SYNTHESIS OF THE NATURAL COUMARINOLIGNOIDS PROPACIN AND CLEOMISCOSIN A AND B. AN EMPIRICAL SPECTROSCOPIC METHOD TO DISTINGUISH REGIOISOMERS OF NATURAL BENZODIOXANE LIGNOIDS

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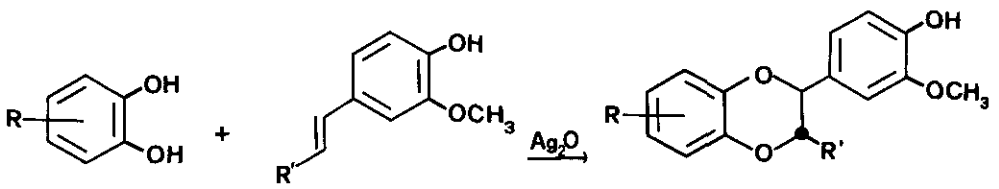
Abstract - The synthesis of the title coumarinolignoids by Ag_2O oxidation of fraxetin and isoeugenol or coniferyl alcohol is reported. A simple diagnostic method based on ^{13}C nmr spectra is proposed to distinguish regioisomers of natural benzodioxane lignoids.

Recently one of us[1] has reported on the one-step synthesis of 2-arylbenzodioxanes by phenol coupling of substituted catechols with para-OH styrenes in the presence

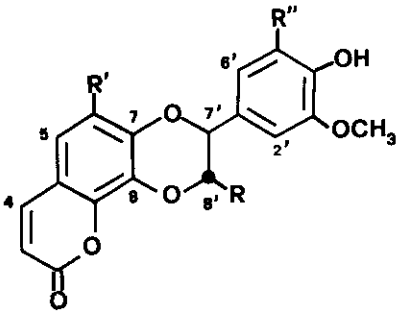


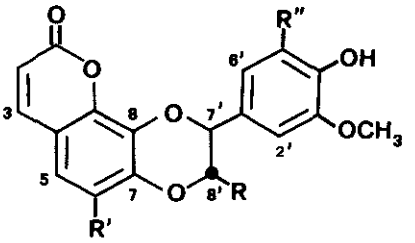
of Ag_2O. This procedure appears particularly useful for the biomimetic synthesis of natural benzodioxane lignoids deriving from similar *in vivo* coupling of isoeugenol or coniferyl alcohol or sinapyl alcohol with catechol containing moieties, such as flavonoids, xanthones or coumarins. Recently elucidated examples of the last class are propacin (Ia or Ib)[2], cleomiscosin A (IIa)[3], cleomiscosin B (IIb)[3], daphneticin (IIIb)[4] and aquillochin (IVa or IVb)[5,6].

We report here a successful application of this method to the synthesis and structural assignment of natural propacin and to the synthesis of both

cleomiscosin A and B.

Oxidation of an equimolar mixture of fraxetin (7,8-dihydroxy-6-methoxycoumarin, V) and isoeugenol in toluene/MeOH with Ag_2O at room temperature afforded 30% of (Ia), accompanied by small amounts of Ib. Comparison of Ia and its acetate (^{13}C nmr, mixed mp) with natural propacin, kindly performed by Prof. O.R. Gottlieb, established their identity and therefore allowed to assign structure Ia (see later) to natural propacin. A certain amount of cis products was shown to be present in the crude reaction mixture by glc and nmr.





Ia	$R = CH_3$	$R' = OCH_3$	R" = H	Ib
IIa	$R = CH_2OH$	$R' = OCH_3$	R" = H	IIb
	$R = CH_2OH$	R' = H	$R" = OCH_3$	IIIb
IVa	$R = CH_2OH$	$R' = OCH_3$	$R" = OCH_3$	IVb

Similar reaction of (V) with coniferyl alcohol gave a mixture of cleomiscosin A (IIa) and B (IIb), also accompanied by some cis derivatives, in ca. 1:1 ratio (glc of TMS ethers). The two trans isomers were separated by flash chromatography on silica gel with AcOEt. Direct comparison (tlc, glc of TMS ether) with authentic samples of cleomiscosin A and cleomiscosin B ethyl ether[7] confirmed the structures.

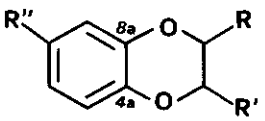
The correct assignment of the structures of regioisomeric compounds of the type a and b of natural or synthetic origin has met considerable difficulty, due to the extreme similarity of the spectral properties, e.g. of ^1H, ^{13}C nmr spectra and of mass spectra, of the compounds of the two classes. In most cases, the problem has been circumvented only by unambiguous but cumbersome chemical synthesis, as in the case of silybin[8] and americanin A[9], or by chemical degradation (for kielcorin[10]). The application of the lanthanide-induced shift method is restricted to particularly favourable structures (e.g. eusiderins[11]). Therefore, final assignment of structure a vs. b was left undecided for some coumarinolignoids

(aquillochin[5], propacin[2]) and neolignans (americanin B[12] and the insect cuticle benzodioxanes[13]) or was made only tentatively (e.g. for xanthonolignoids such as cadensin A[14], cadensin B[14] and cadensin C[15]).

Only very recently a possible simple solution of the problem has been offered by Ray et al.[3], who have reported the selective heteronuclear decoupling of carbons 7 by irradiating H-7', and of carbons 8 by irradiating H-8' respectively, in structures such as a and b.[16] As in most cases carbons 7 and 8 can be assigned on the basis of the structure of the "left" moiety only of A (or B), and H-7' and H-8' have a large difference of chemical shift, a successful decoupling experiment settles the question of the regioisomerism. This method has been also used to establish the structure of daphneticin[4]. It appears, however, that this method has been applied so far only to acetates in $CDCl_3$, most probably for solubility reasons, and we have found that in order to detect without doubt such a very small coupling ($\leq$ 1 Hz) a concentrated solution, and therefore a considerable amount of substance is necessary, and moreover experimental conditions are critical.[17]

We propose here an alternative simple empirical rule, based on systematic differences in ^{13}C chemical shifts of carbons 7 and 8, which has the advantage of requiring small amounts of substance, and no particular solvent, provided that both isomers are available.

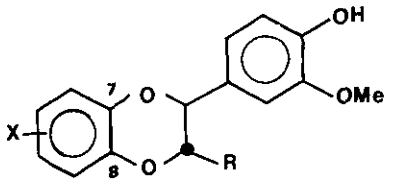
We have observed on some simple benzodioxane models (Fig. 1) that a substituent



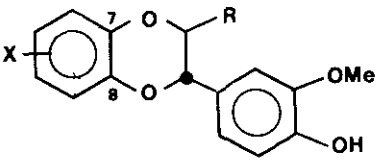
	R	R'	R"	C-4a	C-8a
	H	H	H	143.6	143.6
	CH_3	H	H	143.0	143.4
	CH_2OH	H	H	143.0	142.9
	Ph	H	H	143.0	143.8
(VI)	4-OH-3-OMe-Ph	CH_3	CH_3	141.1	143.6
(VII)	CH_3	4-OH-3-OMe-Ph	CH_3	141.8	143.1

Fig. 1. - ^{13}C chemical shifts for some 1,4-benzodioxanes (2M in $CDCl_3$).

(CH_3, CH_2OH, phenyl) in position 2 induces a small (ca. 0.6 ppm) upfield shift on carbon 4a, whereas carbon 8a is deshielded of ca. 0.2 ppm by a phenyl group but shielded by an alkyl group. Combination of these effects results in a systematic difference of chemical shift of carbons 4a and respectively 8a, depending on the position of the aryl substituent, in trans-2-aryl-3-alkyl-substituted benzodioxanes vs. the 2-alkyl-3-aryl-substituted ones. This is clearly shown in the case of



A



B

		C-7	C-8	$\Delta\delta_{C-7}$	$\Delta\delta_{C-8}$
CLEOMISCOSIN A	Py	138.4	133.0	+0.3	-0.2
CLEOMISCOSIN B	Py	138.1	133.2		
CLEOMISCOSIN A ACETATE	$CDCl_3$	136.9	131.7	+0.6	-0.4
CLEOMISCOSIN B ACETATE	$CDCl_3$	136.3	132.1		
PROPACIN	Py/DMSO	138.5	132.6	+0.7	-0.7
Ib	Py/DMSO	137.8	133.3		
ISOSILYBIN	$CDCl_3$	143.7	142.7	+0.3	-0.4
SILYBIN	$CDCl_3$	143.4	143.1		

Fig. 2. - ^{13}C chemical shifts[16] of regiosomeric natural benzodioxanes.

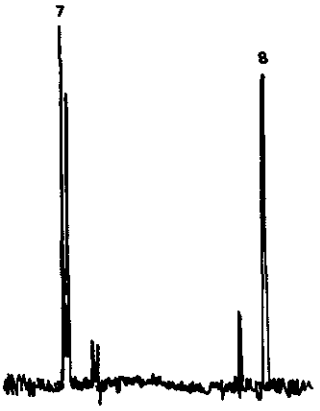


Fig.3 - ^{13}C nmr spectrum (in part) of propacin (major isomer) and Ib. (undecoupled)

the regioisomeric benzodioxanes (VI) and (VII) (Fig. 1), which have been prepared with a regiospecific synthesis.[1] The effect is so small that even a tentative explanation (electronic effect depending on conformational factors?) may be objectionable. However, its systematic appearance in some couples of regioisomeric natural trans-1,4-benzodioxanes (Fig. 2), where the ^{13}C assignments are supported by independent measurements (e.g. selective decoupling[3,1]) lets us suggest its use as a diagnostic method to distinguish these regioisomers. Simple observation of the region of the spectrum where carbons 7 and 8[16] (which must be independently assigned) appear, makes the assignment straightforward, also on mixtures. In the case of propacin (Fig. 3) or cleomiscosins, carbon 7 of isomers A and B are easily assigned because they appear as doublets, due to the meta ^{3}J coupling with the aromatic proton on the coumarin nucleus. On this basis, the structure (Ia) is assigned to the major isomer obtained in the synthesis of propacin, and therefore to natural propacin.

EXPERIMENTAL

Mps are uncorrected. ^{1}H nmr spectra were measured with Bruker WP 80 Sy and CXP 300 instruments, ^{13}C nmr spectra with a Bruker CXP-300 instrument. Chemical shifts are in ppm (δ) from TMS as internal standard. The assignments of signals in ^{13}C nmr spectra are based on heteronuclear selective decoupling. Capillary glcs were performed on a DANI 3800 gas chromatograph using a SP-2100, 30 m x 0.5 mm ID glass column, He flow rate 4.5 ml/min; splitter 120 ml/min, T 280°C. Gas/mass chromatograms were measured with a SE-52, 21 m x 0.25 mm ID fused silica column, He pressure 20 psi, splitter 60 ml/min., T 280°C in a Finnigan 4021 apparatus, with INCOS data system.

Propacin (Ia). 300 mg of fraxetin (V) in 15 ml MeOH and 30 ml toluene were added with 0.6 ml of trans-isoeugenol and 800 mg of Ag_2O (Fluka) and stirred 2 days at room temperature. Filtration, washing with hot $CHCl_3$ and evaporation gave a crude product. Silylation of this mixture with bistrimethylsilyltrifluoroacetamide (BTSFA) in pyridine and glc showed the presence of dehydroisoeugenol, and of three products in 13:21:66 ratio, all with mass: m/e (%): 442(26-30), 236(100), 206(58-62), 205(20-29), corresponding to the structure of a mono-TMS ether of

propacin or of isomers. Flash chromatography on Merck 60 silica gel (17 g) with hexane/AcOEt 1/1 gave 120 mg of (Ia), (third peak to be eluted in the glc of TMS ethers), mp 225-227°C (lit.[2] 226-228°C), ^{1}H nmr: see lit.[2]; ^{13}C nmr (Py-d_5 + DMSO): δ, 17.1 (q, CH_3-9'), 56.0, 56.2 (2q, OCH_3), 74.5 (d, C-8'), 81.5 (d, C-7'), 101.3 (d, C-5), 112.0 (s, C-10), 112.2 (d, C-2'), 113.9 (d, C-3), 116.5 (d, C-5'), 121.5 (d, C-6'), 127.6 (s, C-1'), 132.6 (s, C-8), 138.5 (s, C-7), 139.3 (d, C-9), 144.4 (d, C-4), 146.3 (s, C-6), 149.0 (s, C-4'), 160.7 (s, C-2). The signal of C-3' is masked by the solvent whereas those of the isomer (Ib) appear at 133.3 (C-7) and 137.8 (C-8).

Acetylation of (Ia) (80 mg), with 100 mg AcONa and 3 ml Ac_2O on a steam bath for 6 hr, evaporation, taking up with water and AcOEt, and crystallization from benzene gave 70 mg of (Ia acetate), mp 200-201°C (lit.[2] 202-205°C), ^{1}H nmr see lit.[2]; ^{13}C nmr ($CDCl_3$): δ 17.1 (C-9'), 20.6 (CH_3CO), 56.2 and 56.5 (2 OCH_3), 74.2 (C-8'), 81.1 (C-7'), 100.6 (C-5), 111.8 (C-2'), 111.9 (C-10), 114.2 (C-3), 120.3 (C-6'), 123.3 (C-5'), 132.4 (C-8), 134.7 (C-1'), 137.7 (C-7), 139.0 (C-4'), 140.9 (C-9), 143.6 (C-4), 146.0 (C-6), 151.4 (C-3'), 160.6 (C-2), 168.6 ($COCH_3$), identical with that of the acetate of natural propacin [19].

Signals for the isomer (Ib) (no signal for the cis isomers present in the ^{1}H nmr spectrum), appear at 17.2 (C-9'), 74.8 (C-8'), 80.4 (C-7'), 100.8 (C-5), 120.2 (C-6'), 123.1 (C-5'), 132.7 (C-8), 134.6 (C-1'), 137.1 (C-7), 143.5 (C-4). Attempts to decouple C-7 and C-8 from H-7' and H-8' in both (Ia) and (Ia acetate) according to Ray et al.[3b] were unsuccessful.

Cleomiscosin A (IIa) and B (IIb).

400 mg of fraxetin (V) and 400 mg of coniferyl alcohol were dissolved in 20 ml MeOH and 30 ml toluene and added with 1 g Ag_2O, and the mixture was stirred 24 hr at room temperature. Filtration and evaporation gave a crude product; silylation of this product with BTSFA in pyridine and glc showed the presence of four products in a 25:8:36:30 ratio, all of them showing prominent peaks in the mass spectrum at m/e 530, 440, 324, 293, corresponding to structure (IIa) or isomers. Flash chromatography on Merck 60 silica gel with AcOEt afforded 60 mg of Cleomiscosin A (IIa), mp 257°C (lit.[3] 247-249°C), 300 MHz ^{1}H nmr (py-d_5): δ 3.70 and 3.80 (2 OCH_3), 3.92 (H-9'B, J = 12.9, 3.0), 4.32 (H-9'A, 12.9, 2.2), 4.49 (H-8', 8.0, 2.2, 3.0), 5.60 (H-7', 8.0), 6.45 (H-3, 9.5), 6.74 (H-5), 7.31 (H-5', 8.1), 7.36 (H-6', 8.1, 2.0), 7.43 (H-2', 2.0), 7.75 (H-4, 9.5); ^{13}C nmr (py-d_5): see lit.[3]. The TMS ether (3rd to be eluted in glc) has mass: m/e(%): 530(23), 440(56), 324(92), 293(40), 73(100), and is indistinguishable in glc from a sample of authentic Cleomiscosin A[7]; further elution gave 30 mg of Cleomiscosin B (IIb), mp 275-278°C (lit.[3] 274°C), 300 MHz ^{1}H nmr (py-d_5): δ 3.72 and 3.84 (2 OCH_3),

3.94 (H-9'A, J = 12.8, 3.5), 4.29 (H-9'B, 12.8, 2.3), 4.54 (H-7', 8.0, 2.3, 3.5), 5.55 (H-8', 8.0), 6.42 (H-3, 9.5), 6.76 (H-5), 7.31 (H-5', 8.1), 7.36 (H-6', 2.0, 8.1), 7.44 (H-2', 2.0), 7.75 (H-4, 9.5); ^{13}C nmr (py-d_5): see lit.[3]. The TMS ether (4th to be eluted in glc) has mass m/e (%): 530(34), 440(23), 324(90), 293(45), 73(100). Nmr of other, impure fractions showed the presence of cis isomers, as it appears from the signal of H-7' at 5.70 (d, J 3) in pyridine-d_5.

2-Phenyl-1,4-benzodioxane. - 2-Hydroxy-2-phenyl-1,4-benzodioxane[20] (2g) was reduced with 0.6 g $NaBH_4$ in EtOH at room temp. After workup 2-(2-hydroxy)phenoxy)-1-phenylethanol (1.8 g) was obtained, mp. 98°C (cyclohexane), anal.: Found: C,73.03; H,6.07; calcd. for $C_{14}H_{14}O_3$: C, 73.02; H, 6.13; nmr ($CDCl_3$): δ 4.10, 4.15 (CH_2-O), 5.13 (CH-O), 6.8-7.1 (4H), 7.4 (5H); ms: m/e(%) 230(11), 212(44), 121(25), 110(100). This compound (0.9 g) was refluxed 1 hr in toluene with 0.2 g of Amberlyst 15. Filtration, washing with CH_2Cl_2 and chromatography gave 0.4 g of 2-phenyl-1,4-benzodioxane, mp. 39-40°C, Anal.: Found: C, 79.38; H, 5.71; calcd. for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70; nmr (2M in $CDCl_3$): δ 3.85 (H-3A, J = 12, J =9 Hz), 4.16 (H-3B, 12,3), 4.93 (H-2, 3,9), 6.6-7.0 (4H, Aryl)), 7.3 (5H, Ph).

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16.For sake of uniformity, the numbering used by Ray et al.[3] for cleomiscosins has been adopted.

17.In our hands this experiment was unsuccessful on propacin (in py-d_5) and propacin acetate, and showed a very small sharpening of signals in the case of silybin pentaacetate (300 mg/1.5 ml $CDCl_3$).

18.A detailed analysis of the ^{13}C nmr spectra of these compounds shall appear elsewhere. The chemical shifts given here were obtained by measuring the spectra on 2M solutions in $CDCl_3$.

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