Synthesis and sweet taste of optically active (−)-haematoxylin and of some (±)-haematoxylin derivatives

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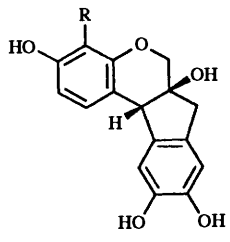
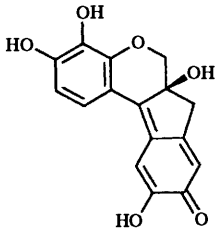
In order to explain the sweet taste of the natural polyphenolic compound (+)-haematoxylin **1**, four (±)-haematoxylin derivatives **4–7** and the enantiomer (−)-haematoxylin have been synthesized and tasted. Unlike haematoxylin, the derivatives **4–7** have a restricted number of different possibilities of binding to the sweet taste receptor according to the Shallenberger–Acree–Kier model. This allowed the study of the most likely orientation of these compounds in the active site of receptor. The results are supported by the comparison of the molecular structures with the receptor models of Temussi–Goodman and Tinti–Nofre. The synthesis of the (−)-enantiomer of haematoxylin also allows for discussion of the relationship between configuration and sweet taste in these compounds.

(+)-Haematoxylin **1** is a long-known natural compound extracted from the plant *Haematoxylon campechianum* (Leguminosae). It is easily oxidized by air to haematein **2** and therefore it is used in histology as a dye.[1] Recently some authors[2] identified **1** as the substance responsible for the sweetness of the plant extracts. The analogous compound brazilin **3** has been reported by the same authors to be tasteless[2] although in older literature it is said to be sweet[3] with a taste potency of 100 × sucrose.[4]



(+)-1 R = OH
(+)-3 R = H



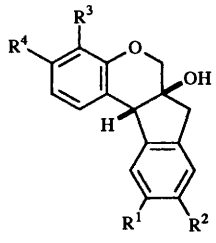
(+)-2

Our interest in haematoxylin and in the mechanism by which it interacts with the sweet substances receptor arises from several considerations.

(1) This molecule has a marked structural similarity with the isovanillic sweeteners studied by us.[5] In fact, vicinal hydroxy groups in alcohols and phenols can act as the AH-B binding site in the Shallenberger–Acree model[6] of the sweet receptor. In this model one of the hydroxyls acts as a H-bonding donor and the other as an acceptor. A similar interaction is more often and more effectively obtained with an *ortho* hydroxy-methoxy system, *e.g.* in the class of isovanillic sweeteners.

(2) Compound **1** is extremely rigid from a conformational point of view owing to its polycyclic structure. In structure–taste correlations, much work has been devoted to the resolution of conformational problems owing to the fact that flexible sweet substances can adopt many possible conformations in the interaction with the receptor, only one of them being the active one. In this respect, the examination of rigid sweet substances such as **1** has the advantage of giving unambiguous geometric relationships between groups.

(3) Natural haematoxylin is an optically active compound; it



Compd.	R^1	R^2	R^3	R^4	Orientation
4	OH	OMe	H	H	**F, D**
5	OMe	OH	H	H	**E, D**
6	OMe	OMe	OH	OMe	**A, C, D**
7	OMe	OMe	OMe	OH	**B, D**
(+)-**8**	OMe	OMe	OMe	OMe	**D**

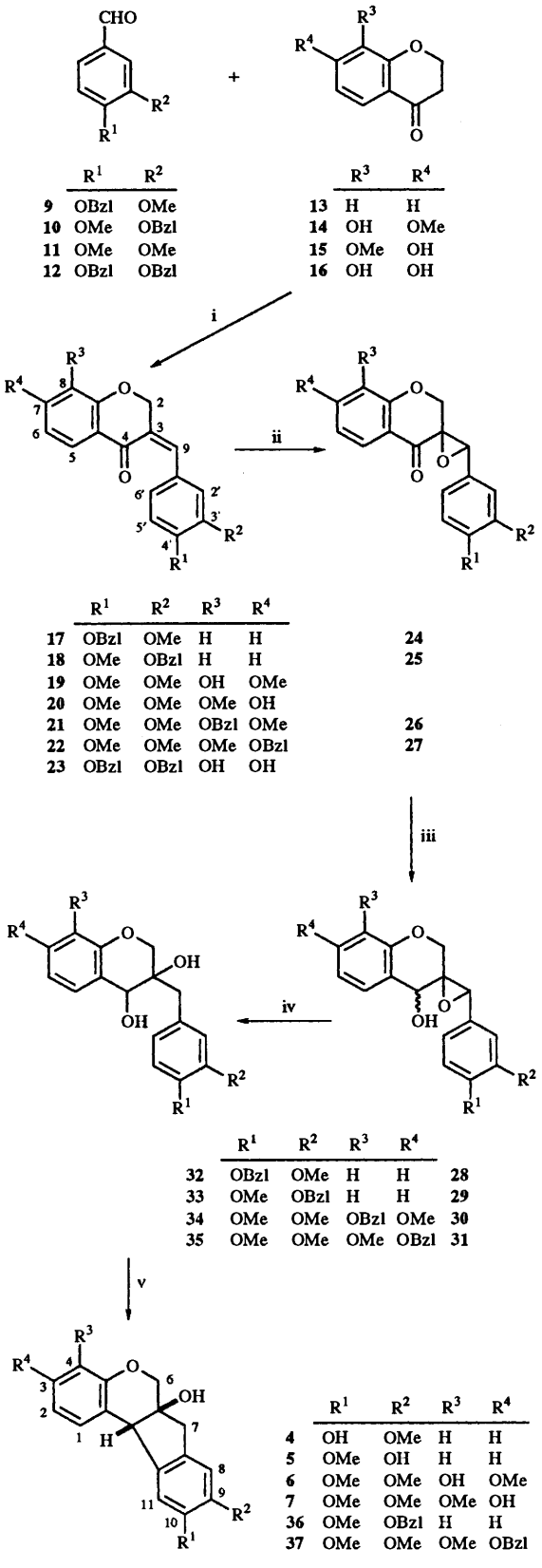
Fig. 1 Chemical structure and possible orientations of compounds **4–8**

is a pentahydroxydihydrobenzindenopyran with a *cis* junction between the cyclopentane and pyran rings and a 6a*S*,11b*R* configuration[7]. The interactions of chiral molecules with the receptor are different for the two enantiomers and stereochemistry has been often used in designing receptor shape.

Therefore we have synthesized the enantiomer of haematoxylin [compound (−)-**1**] and four racemic haematoxylin derivatives (compounds **4**, **5**, **6** and **7**) (Fig. 1); the new compounds were tasted and their structures compared with the existing receptor models.

Results

Synthesis of compounds **4–7**. The general synthetic scheme developed by Dann and Hofmann[8] for (±)-haematoxylin was followed (Scheme 1). The first step was the acid-catalysed condensation between an aldehyde (**9–11**) and a chromanone (**13–15**) to give compounds **17–20**. Selective methylation of 7,8-dihydroxychroman-4-one **16** was achieved taking advantage of the different reactivity of the two phenolic groups. In the presence of a mild base such as potassium carbonate, the 7-OH is selectively methylated owing to its higher acidity;[9] whereas with 2 equivalents of the strong base NaH methylation occurs predominantly at the 8-OH which is more reactive in the phenoxide form.[10] Compounds **19** and **20** were protected as benzyl ethers **21** and **22** prior to the second reaction step, *i.e.* the epoxidation to epoxy ketones **24–27**. These were reduced to the epoxy alcohols **28–31** with $NaBH_4$; subsequent treatment with $LiAlH_4$ gave the diols **32–35**. Cyclisation to the final products was effected by $HClO_4$; under these conditions, derivatives **32** and **34** afforded directly the debenzylated products **4** and **6** respectively, whereas **33** and **35** gave the benzylated compounds



	R^1	R^2
9	OBzl	OMe
10	OMe	OBzl
11	OMe	OMe
12	OBzl	OBzl

	R^3	R^4
13	H	H
14	OH	OMe
15	OMe	OH
16	OH	OH

	R^1	R^2	R^3	R^4
17	OBzl	OMe	H	H
18	OMe	OBzl	H	H
19	OMe	OMe	OH	OMe
20	OMe	OMe	OMe	OH
21	OMe	OMe	OBzl	OMe
22	OMe	OMe	OMe	OBzl
23	OBzl	OBzl	OH	OH

24 **25** **26** **27**

	R^1	R^2	R^3	R^4	
32	OBzl	OMe	H	H	**28**
33	OMe	OBzl	H	H	**29**
34	OMe	OMe	OBzl	OMe	**30**
35	OMe	OMe	OMe	OBzl	**31**

	R^1	R^2	R^3	R^4
4	OH	OMe	H	H
5	OMe	OH	H	H
6	OMe	OMe	OH	OMe
7	OMe	OMe	OMe	OH
36	OMe	OBzl	H	H
37	OMe	OMe	OMe	OBzl

Scheme 1 *Reagents:* i, HCl gas; ii, H_2O_2, NaOH; iii, $NaBH_4$; iv, $LiAlH_4$; v, $HClO_4$

36 and **37** which were then deprotected by catalytic hydrogenation to the final derivatives **5** and **7**. The presence of the activating methoxy substituents R^1 and R^2 appeared essential for the cyclization to occur. This precluded the synthesis, by this method, of the analogues with the D ring unsubstituted.

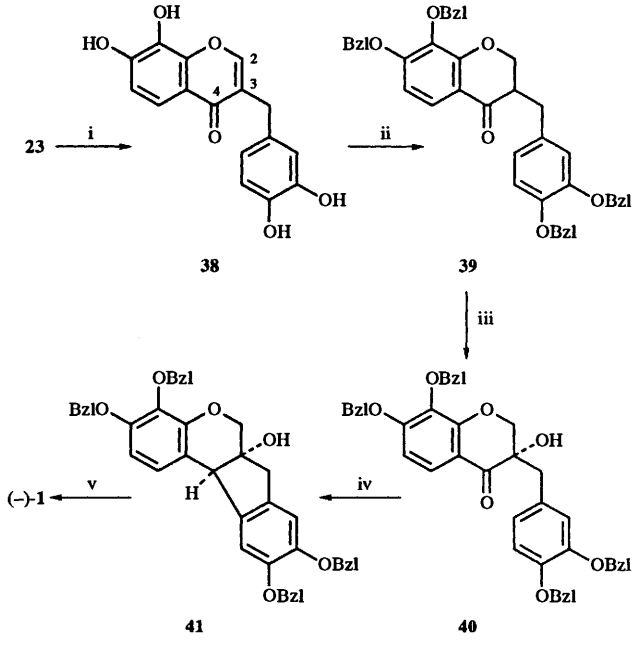
Table 1 Taste potency of compounds

Compound	(+)-**1**	(−)-**1**	**4**	**5**, **6** and **7**	**8**
Taste*	sweet 120×	sweet 50×	sweet 50×	tasteless	bitter

* Relative to a 3% aqueous solution of sucrose as reference.

Synthesis of (−)-haematoxylin 1

The acid-catalysed condensation of the aldehyde **12** and chromanone **16** gave the unsaturated compound **23** which was hydrogenated to **38** and again protected as the tetrabenzyl derivative **39**. The enantioselective introduction of the tertiary hydroxyl in position 3 was obtained with (−)-8,8-dichlorocamphorylsulfonyloxaziridine, following the procedure reported for the asymmetric synthesis of (+)-*O*-trimethylbrazilin[11] (Scheme 2). In this case the expected

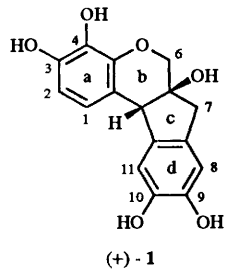


Scheme 2 *Reagents:* i, H_2, Pd/C; ii, $PhCH_2Br$, K_2CO_3; iii, NaHMDS; iv, (−)-8,8-dichlorocamphorylsulfonyloxaziridine; v, $NaBH_4$; vi, HCl; vii, H_2, Pd/C

configuration is 3*S*, which is needed to obtain the enantiomer of the natural product. The alcohol (−)-**40** which was obtained in 42% yield, was reduced with $NaBH_4$ and cyclised with HCl in one step to (−)-haematoxylin tetrabenzyl ether **41**. The optical purity of this compound was 99% by $[\alpha]_D$ measurements and a CLSR NMR experiment. Catalytic hydrogenation afforded optically pure (−)-haematoxylin in 20% yield.

Tasting of compounds

(+)-Haematoxylin **1** is reported to be sweet but a quantitative value is not given in the literature.[2] This compound, its enantiomer (−)-**1** and compounds **4**–**7** were tasted by an untrained voluntary panel of seven persons as diluted aqueous solutions in comparison with a 3% sucrose solution following a standard procedure.[12] Optically active (+)-tetramethylhaematoxylin **8**,[13] synthesized from natural haematoxylin, was also tasted. The results are showed in Table 1.



(+)-1

Groups position AH	B	Orientation
4	3	A
3	4	B
4	5	C
6a	5	D
9	10	E
10	9	F

Fig. 2 Possible orientations of compound (+)-**1** in the interaction with the receptor. Hydroxyls can act as both AH and B groups; in orientations C and D AH is an hydroxyl, B is the ethereal oxygen.

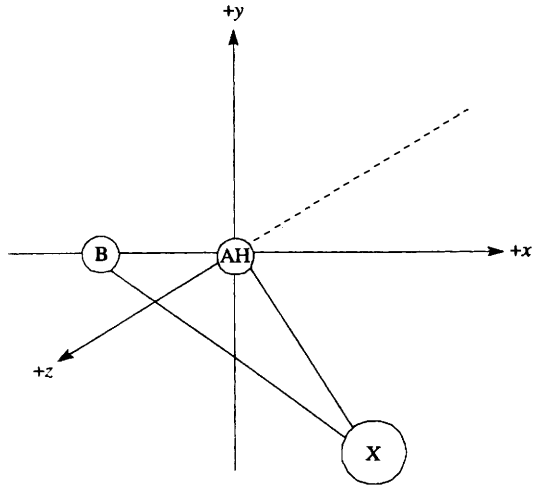


Fig. 3 Topological arrangement of the glucophores AH, B and X in sweet molecules.

Discussion

In order to explain the sweet taste of haematoxylin and its derivatives, the possible interactions of these molecules with the receptor have to be considered.

Compound **1**, owing to the presence of five OH groups of the 3-, 4-, 6a-, 9- and 10-positions and the ethereal oxygen at the 5-position, has six possible ways to give an AH-B interaction with the complementary site of the receptor. In other words, there are six possible orientations of the molecule in the receptor active site (Fig. 2). On the other hand, the methoxy derivatives **4**–**7** can assume only one (**4**, **5**, **7**) or two (**6**) possible orientations besides orientation **D**, this last being the only one pertaining to **8**.

Having determined which groups act as the AH-B system, the overall efficiency of the interaction, and thus the taste potency of the compound, will depend also on the resulting position of the other binding groups, mainly of the hydrophobic X group. This was first identified by Kier[14] and is sometimes referred to as G group. Its importance in giving sweetness has been recognized also in more recent models such as those by Temussi,[15] Goodman,[16] and Tinti–Nofre.[17] The relative positions of these glucophores are therefore important. To understand which is the actual orientation of **1** in receptor interactions, we matched the six orientations **A**–**F** with the models using a molecular mechanics program; the results were compared to experimental evidence obtained by tasting compounds **4**–**8**, each of them representing only a few possible orientations of haematoxylin.

Fig. 3 shows the qualitative topological arrangement of the glucophores AH-B and X. In sweet substances, these points are the vertices of a triangle whose average distances are critical.

In Fig. 3 the origin of the cartesian coordinates has been chosen arbitrarily in order to facilitate comparison of our molecules with other sweet substances and with models. In fact, the presence of this moiety has been recognized in several classes of sweet molecules, but often literature data differ in the choice of the geometric reference system. For this reason, it is often difficult to compare precisely molecules and models, different molecules with each other and even the same molecule minimized with different molecular mechanics and computer graphics programs.

Therefore we put the origin of the cartesian system into AH (AH = 0, 0, 0), so that B lies on the negative x axis (B = $-x$, 0, 0), and the X group in the x, y plane. This topological definition is analogous to that proposed by Goodman[16] for the classification of sweet peptides into five classes, but in that case the origin of the cartesian coordinates system was located at the α-carbon of the second amino acid residue, so that a direct comparison with non-peptide sweeteners is not obvious.

In this system, a simple requirement for a molecule to elicit sweetness is that X must reside in the $+x$, $-y$ semiplane, *i.e.* X = x, $-y$, 0. This condition corresponds to the geometric requirement that the distance B-X is > the AH-X, and it is necessary (but not sufficient) to elicit sweetness for molecules having these three receptor binding groups. The actual position of X, which in some way reflects the 'inclination' of the molecule is critical and it is also more difficult to define because X is usually a molecular fragment rather than an atom. Anyway, owing to its importance, it has been extensively discussed by Goodman for sweet peptides;[16] a similar qualitative criterion to establish the relative position of this group has been proposed by us for sweet isovanillic heterocycles.[12] For these compounds also, the angle between the isovanillic system (AH-B) and the lipophilic site must be in a definite range of values for the molecule to be sweet.

The molecular structure of (+)-**1** was minimized with the MMPM1 molecular mechanics program and then superimposed onto one of the existing models in each of the six possible orientations **A**–**F**. The centre of the X group was arbitrarily chosen as the carbon atom of the aromatic ring opposite to that containing the AH-B system giving the best AH-X and B-X distances. The best fit with the model was obtained qualitatively by docking the molecule to the model and minimizing the distances between the three bonding pairs.

Table 2 reports the coordinates of (+)-haematoxylin in each of the orientations **A**–**F**. For a qualitative comparison, the coordinates of aspartame in the 'L shaped' conformation were reported as they could be obtained from the literature.[16] The coordinates of the Tinti–Nofre hypersweet model[17] were also used for comparison.

The basic requirement for sweetness (*i.e.* X = $+x$, $-y$, 0) is only satisfied in orientations **A** and **F**. Moreover, in orientation **F**, the relative position of the X group is much more similar to that of Goodman and Tinti–Nofre models (see Table 2).

These results are in agreement with those obtained by tasting the new haematoxylin derivatives. In fact, compounds **5**–**8**, which could adopt only orientations **E** and **D**, **B** and **D**, and **D** respectively, are tasteless or bitter and this is in agreement with the fact that in orientations **B**, **C**, **D** and **E** the basic requirement is not satisfied. The fact that compound **4** (which adopts the **F** or **D** orientation) is sweet, whereas compound **6** (which has the **A** or **D** orientation) is tasteless, seems to indicate that the most probable orientation for haematoxylin and its derivatives is that represented by **F**.

The direct comparison of (+)-**1** in the **F** orientation with the Goodman model for aspartame and the Tinti–Nofre model is shown in Fig. 4.

The simple topological analysis described here cannot discriminate between (+)- and (−)-haematoxylin, which have

Table 2 Cartesian coordinates of (+)-**1** in the six possible orientations[a]

Compound	Orientation	x(B)	x(X)	y(X)	Identity (X)
	A	−2.77	0.09	−8.40	C 9
	B	−2.77	−1.66	−7.69	C 10
(+)-**1**	**C**	−2.73	−5.82	−5.16	C 10
	D	−2.89	−1.27	−5.79	C 10
	E	−2.77	−5.01	−7.07	C 4
	F	−2.77	2.24	−7.07	C 4
Aspartame[b]		−3.14	4.31	−3.13	
Tinti–Nofre model[c]		−2.79	3.28	−5.20	

[a] (x, y, z)(AH), (y, z)(B) and z(X) = 0 for every compound. [b] Calculated from ref. 16; AH is N of $-NH_3^+$, B is C of COO^-, X is one carbon *meta* of Phe ring. [c] From ref. 17 by translating the origin in AH.

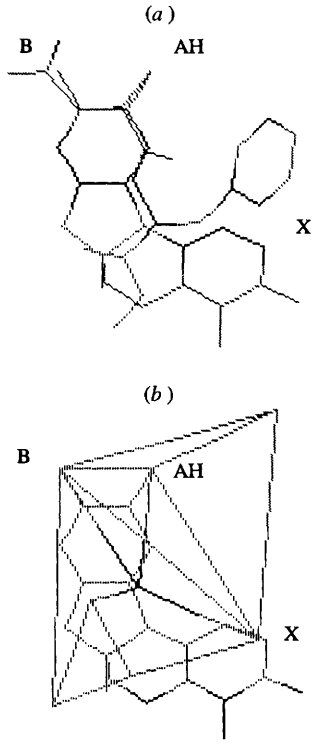


Fig. 4 Superimposition of compound (+)-**1** with (*a*) aspartame and (*b*) the Tinti–Nofre model.

a flat bowl-shape due to the *cis* ring junction. For this purpose, it would be necessary to define at least another point lying outside the x, y plane. Comparison with the Goodman and Tinti–Nofre models does not support there being sharp differences between the enantiomers, since in these molecules it is difficult to identify other possible binding sites like D, Y, E_1, E_2, XH. On the other hand both molecules are sweet, and the difference in taste potency is too small to draw any conclusion on this point.

In conclusion, an analysis of sweet receptor models, together with the synthesis of suitable derivatives, suggests that the hydroxyls in the 10, 9 position of (+)-haematoxylin **1** are important in their interaction with the receptor, probably acting as the AH-B moiety. Structural differences between (+)-**1** and its enantiomer on interaction with the receptor are not discriminating, owing to the fact that the receptor is little defined outside the x, y plane, which is the mirror plane for this class of molecules. Further studies on substances with rigid molecular structures, particularly with regard to the stereochemistry, should help to further detailed understanding of the structure–taste relationships for these compounds.

Experimental

^{1}H NMR spectra were recorded on a Bruker WP80SY (80 MHz) and a Varian XL300 (300 MHz) spectrometer with Me_4Si as internal standard; chemical shift values are in δ (ppm); J values are given in Hz. Mass spectra (electron impact) were recorded with a Finnigan-MAT TSQ70 spectrometer with an ICIS data system. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mps are uncorrected. Flash chromatography was performed on silica gel Merck 60 (230–400 mesh STM). Compounds **5** and **9**–**11** are commercial (Aldrich). (−)-(8,8-Dichlorocamphorylsulfonyl)oxaziridine was purchased from Fluka.

7,8-Dihydroxy-2,3-dihydro-4*H*-1-benzopyran-4-one 16. Beige solid, mp 189 °C (lit.,[18] 188–189.5 °C); δ_H($CDCl_3$–[$^2H_6$]-acetone) 2.78 (2 H, t, J 7, 3-H), 4.60 (2 H, t, J 7, 2-H), 6.50 (1 H, d, J 9, 6-H), 6.75 (OH), 7.41 (1 H, d, J 9, 5-H) and 7.75 (OH).

8-Hydroxy-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one 14. A mixture of compound **16** (2.2 g, 12 mmol), K_2CO_3 (1.9 g, 14 mmol) and MeI (0.83 cm^3, 13 mmol) in DMF (20 cm^3) was stirred at 55 °C for 5 h and then filtered, acidified with hydrochloric acid and extracted with ethyl acetate. Flash chromatography with hexane–ethyl acetate (6:4) gave **14** as a yellow solid (0.72 g, 31% yield); mp 123 °C (Found: C, 61.7; H, 5.2. $C_{10}H_9O_4$ requires C, 61.85; H, 5.19%); δ_H($CDCl_3$) 2.80 (2 H, A_2 of A_2B_2, 3-H), 3.95 (3 H, s, OCH_3), 4.61 (2 H, B_2 of A_2B_2, 2-H), 5.45 (OH), 6.63 (1 H, d, J 9, 6-H) and 7.53 (1 H, d, J 9, 5-H).

7-Hydroxy-8-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one 15. NaH (80% in paraffin oil; 0.72 g, 24 mmol) and MeI (0.68 cm^3, 11 mmol) were added to a solution of compound **13** (2 g, 11 mmol) in DMF (20 cm^3). After being stirred at room temperature for 24 h, the reaction mixture was evaporated, acidified with hydrochloric acid and extracted with ethyl acetate. Flash chromatography with hexane–ethyl acetate (1:1) gave **15** as a white solid (0.4 g, 19% yield), mp 155 °C; δ_H($CDCl_3$) 2.75 (2 H, t, J 6, 3-H), 3.95 (3 H, s, OCH_3), 4.5 (2 H, t, J 6, 2-H), 6.2 (1 H, s, OH), 6.62 (1 H, d, J 8, 5-H) and 7.76 (1 H, d, J 8, 7-H).

Condensation between aldehydes and 2,3-dihydro-4*H*-1-benzopyran-4-ones

Equimolar amounts of the appropriate aldehyde and chromanone were dissolved in absolute ethanol (20 cm^3 $mmol^{-1}$). The reaction mixture was cooled to 0 °C with an ice bath and HCl gas was bubbled into the reaction mixture for 20 min. The reaction mixture was stirred at room temperature overnight, concentrated, taken up with ethyl acetate, and the solution washed with water, dried and evaporated to dryness.

3-(4-Benzyloxy-3-methoxybenzylidene)-2,3-dihydro-4*H*-1-benzopyran-4-one 17. Flash chromatography with hexane–ethyl acetate (75:25) gave an orange solid (3.3 g, 66%), mp 110 °C (ethyl acetate) (Found: C, 77.2; H, 5.7. $C_{24}H_{20}O_4$ requires C,

77.40; H, 5.41%); $\delta_H(CDCl_3)$ 3.90 (3 H, s, OCH_3), 5.20 (2 H, s, OBz), 5.4 (2 H, d, *J* 2, 2-H), 6.8–7.6 (11 H, m, arom), 7.8 (1 H, t, *J* 2, 9-H) and 8.0 (1 H, dd, *J* 8 and 2, 5-H); *m/z* 372 (M^+, 13%), 281 (17), 137 (20), 131 (11) and 91 (100).

3-(3-Benzyloxy-4-methoxybenzylidene)-2,3-dihydro-4*H*-1-benzopyran-4-one 18. Yellow solid that precipitates from ethanol (11.6 g, 88%), mp 133–134 °C (Found: C, 77.45; H, 5.35. $C_{24}H_{20}O_4$ requires C, 77.40; H, 5.41%); $\delta_H(CDCl_3)$ 3.96 (3 H, s, OCH_3), 5.22 (4 H, m, 2-H and OBz), 6.8–7.52 (11 H, m, arom), 7.75 (1 H, t, 9-H) and 8.0 (1 H, dd, 5-H); *m/z* 372 (M^+, 15%), 281 (44), 221 (4) and 91 (100).

3-(3,4-Dimethoxybenzylidene)-8-hydroxy-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one 19. Yellow solid that precipitates from the reaction mixture (ethanol) (2.96 g, 83%), mp 128 °C; $\delta_H(CDCl_3)$ 3.90 and 3.92 (3 × 3 H, s, OCH_3), 5.4 (1 H, s, OH), 5.45 (2 H, d, *J* 2, 2-H), 6.65 (1 H, d, *J* 9, 6-H), 6.8–7.0 (3 H, m, 2'-, 5'- and 6'-H), 7.65 (1 H, d, *J* 9, 5-H) and 7.82 (1 H, t, *J* 2, 9-H).

3-(3,4-Dimethoxybenzylidene)-7-hydroxy-8-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one 20. Flash chromatography with hexane–ethyl acetate (1 : 1) gave **20** as a yellow solid (1.2 g, 79%), mp 122 °C; $\delta_H(CDCl_3)$ 3.95 (3 × 3 H, s, OCH_3), 5.45 (2 H, d, *J* 2, 2-H), 6.2 (1 H, s, OH), 6.67 (1 H, d, *J* 9, 6-H), 6.8–7.0 (3 H, m, 2'-, 5'-, 6'-H), 7.75 (1 H, d, *J* 9, 5-H) and 7.8 (1 H, t, *J* 2, 9-H).

Protection of free phenolic groups

Compounds **19** and **20** were refluxed with benzyl bromide (1 mmol $mmol^{-1}$) and K_2CO_3 (3 mmol $mmol^{-1}$) in acetone to give the corresponding benzylated compounds **21** and **22**. After 4 h at reflux, the mixture was filtered and concentrated.

8-Benzyloxy-3-(3,4-dimethoxybenzylidene)-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one 21. Yellow amorphous solid (3.7 g, 98%) used without further purification; $\delta_H(CDCl_3)$ 3.9–4.0 (3 × 3 H, s, OCH_3), 5.1 (2 H, s, OBz), 5.35 (2 H, d, *J* 2, 2-H), 6.7 (1 H, d, *J* 9, 6-H), 6.8–7.0 (3 H, m, 2'-, 5'-, 6'-H), 7.2–7.6 (5 H, m, arom), 7.8 (1 H, d, *J* 9, 5-H) and 7.8 (1 H, t, *J* 2, 9-H).

7-Benzyloxy-3-(3,4-dimethoxybenzylidene)-8-methoxy-2,3-dihydro-4*H*-1-benzopyran-4-one 22. Yellow solid (1.28 g, 85%), mp 176 °C; $\delta_H(CDCl_3)$ 3.85–3.92 (3 × 3 H, s, OCH_3), 5.2 (2 H, s, OBz), 5.45 (2 H, d, *J* 2, 2-H), 6.2 (1 H, d, *J* 9, 6-H), 6.87 (3 H, m, 2'-, 5'-, 6'-H), 7.4 (5 H, m, arom), 7.75 (1 H, d, *J* 9, 5-H) and 7.8 (1 H, t, *J* 2, 9-H).

Epoxidation

To a solution of the substrate in methanol–acetone (1 : 1; 50 cm^3 $mmol^{-1}$ of substrate), aqueous NaOH (6 mol dm^{-3}; 6 mmol $mmol^{-1}$) was added; this was followed by H_2O_2 (30%; 3.5 cm^3 $mmol^{-1}$), added dropwise very slowly, the temperature being kept < 30 °C. When the reaction was complete (TLC; after 5–11 h at room temperature) the reaction mixture was diluted with water and the product recovered by filtration.

3'-(4-Benzyloxy-3-methoxy)spiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-one 24. White solid (2.2 g, 71%), mp 115 °C (Found: C, 73.9; H, 5.2. $C_{24}H_{20}O_5$ requires C, 74.21; H, 5.19%); $\delta_H(CDCl_3)$ 3.90 (3 H, s, OCH_3), 4.15 (1 H, d, *J* 12, 2a-H), 4.5 (1 H, s, 3'-H), 4.58 (1 H, d, *J* 12, 2b-H), 5.19 (2 H, s, OBz), 6.88–7.64 (11 H, m, arom) and 8.0 (1 H, dd, *J* 8 and 2, 5-H); *m/z* 388 (M^+, 15%), 296 (19), 269 (7), 236 (8) and 91 (100).

3'-(3-Benzyloxy-4-methoxy)spiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-one 25. White solid (0.2 g, 80%), mp 118–119 °C (Found: C, 74.1; H, 5.3. $C_{24}H_{20}O_5$ requires C, 74.21; H, 5.19%); $\delta_H(CDCl_3)$ 3.90 (3 H, s, OCH_3), 3.95 (1 H, d, *J* 12, 2a-H), 4.45 (1 H, d, *J* 12, 2b-H), 4.50 (1 H, s, 3'-H), 5.20 (2 H, s, OBz), 6.8–7.6 (11 H, m, arom), 7.95 (1 H, dd, *J* 8 and 2, 5-H); *m/z* 388 (M^+, 11%), 297 (13), 269 (10), 173 (4), 151 (6) and 91 (100).

8-Benzyloxy-3'-(3,4-dimethoxybenzyl)-7-methoxyspiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-one 26. White solid (3.0 g, 78%), mp 79–80 °C (Found: C, 69.5; H, 5.2. $C_{26}H_{24}O_7$ requires C, 69.63; H, 5.39%); $\delta_H(CDCl_3)$ 3.8–4.0 (3 × 3 H, s, OCH_3), 4.1 (1 H, d, *J* 12, 2a-H), 4.4 (1 H, d, *J* 12, 2b-H), 4.5 (1 H, s, 3'-H), 5.0 (2 H, s, OBz), 6.7 (1 H, d, *J* 9, 6-H), 6.8–6.9 (3 H, m, 2'-, 5'-, 6'-H), 7.2–7.4 (5 H, m, arom), 7.75 (1 H, d, *J* 9, 5-H); *m/z* 448 (M^+, 11%), 357 (10), 297 (7), 259 (14), 165 (9) and 91 (100).

7-Benzyloxy-3'-(3,4-dimethoxybenzyl)-8-methoxyspiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-one 27. White solid (0.54 g, 67%), mp 50 °C (Found: C, 69.7; H, 5.4. $C_{26}H_{24}O_7$ requires C, 69.63; H, 5.39%); $\delta_H(CDCl_3)$ 4.25 (1 H, d, *J* 13, 2a-H), 4.50 (1 H, s, 3'-H), 4.6 (1 H, d, *J* 13, 2b-H), 5.2 (2 H, s, OBz), 6.75 (1 H, d, *J* 9, 6-H), 6.8–7.0 (3 H, m, 2'-, 5'-, 6'-H), 7.4 (5 H, m, arom) and 7.7 (1 H, d, *J* 9, 5-H).

Reduction of ketones

The substrate was suspended in a mixture of 15 cm^3 $mmol^{-1}$ of isopropyl alcohol and water (4%), and $NaBH_4$ (2.5 mmol $mmol^{-1}$ of substrate) was added in four portions during 6 h. After this time the reaction mixture was diluted with water, concentrated, extracted with ethyl acetate and the extract evaporated to dryness.

3'-(4-Benzyloxy-3-methoxybenzyl)spiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-ol 28. Flash chromatography with hexane–ethyl acetate (7 : 3) gave **28** as a yellowish solid (0.83 g, 39%), mp 210–215 °C (Found: C, 73.6; H, 5.9. $C_{24}H_{22}O_5$ requires C, 73.83; H, 5.68%); $\delta_H(CDCl_3)$ 2.22 (1 H, d, OH), 3.9 (3 H, s, OCH_3), 4.08 (2 H, s, 2-H), 4.40 (1 H, s, 3'-H), 4.95 (1 H, d, *J* 9, 4-H), 5.20 (2 H, s, OBz) and 6.8–7.6 (12 H, m, arom); *m/z* 390 (M^+, 24%), 148 (5), 147 (3) and 91 (100).

3'-(3-Benzyloxy-4-methoxybenzyl)spiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-ol 29. Colourless oil which solidified on treatment with diethyl ether to a white solid (0.44 g, 46%), mp 54–55 °C (Found: C, 73.7; H, 5.6. $C_{24}H_{22}O_5$ requires C, 73.83; H, 5.68%); $\delta_H(CDCl_3)$ 2.15 (1 H, d, OH), 3.8 (1 H, *J* 12, 2a-H), 3.9 (3 H, s, OCH_3), 4.16 (1 H, d, *J* 12, 2b-H), 4.4 (1 H, s, 3'-H), 4.65 (1 H, d, *J* 4, 4-H), 5.18 (2 H, s, OBz) and 6.8–7.5 (12 H, m, arom); *m/z* 390 (M^+, 4%), 271 (23), 243 (14), 148 (44), 137 (8) and 91 (100).

8-Benzyloxy-7-methoxy-3'-(3,4-dimethoxybenzyl)spiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-ol 30. Flash chromatography with hexane–ethyl acetate (1 : 1) gave **30** as a white solid (0.8 g, 53%), mp 53–55 °C; $\delta_H(CDCl_3)$ 3.8–3.9 (3 × 3 H, s, OCH_3), 3.9–4.5 (4 H, m, 2-H_2, 4-H,OH), 5.0 (2 H, s, OBz), 6.6 [1 H, d, *J* 8, 5(6)-H], 6.9 (3 H, br, 2'-, 5'- and 6'-H), 7.1 [1 H, d, *J* 8, 6(5)-H] and 7.2–7.5 (5 H, m, arom).

7-Benzyloxy-3'-(3,4-dimethoxybenzyl)-8-methoxyspiro[2,3-dihydro-4*H*-1-benzopyran-3,2'-oxiran]-4-ol 31. Yellowish amorphous solid used without further purification (0.52 g, 99%); $\delta_H(CDCl_3)$ 3.90 (9 H, s, OCH_3), 4.1 (1 H, d, *J* 13, 2a-H), 4.4 (1 H, d, *J* 13, 2b-H), 4.0–4.5 (3 × 1 H, 3'-H, 4-H, OH), 5.20 (2 H, s, OBz), 6.5–6.6 [1 H, d, *J* 9, 5(6)-H], 6.95 [1 H, d, *J* 9, 6(5)-H], 6.9 (3 H, m, 2'-H, 5'-H, 6'-H) and 7.3–7.5 (5 H, arom).

Reduction of oxiranes

A solution of the substrate in THF was added dropwise to a suspension of $LiAlH_4$ (0.4 mmol $mmol^{-1}$ of substrate) in THF. The reaction mixture was refluxed for 5 h after which 8% aqueous NaOH was added to it (1.8 cm^3 $mmol^{-1}$ of substrate); the solution was then filtered, dried and evaporated to dryness.

3-(4-Benzyloxy-3-methoxybenzyl)-2,3-dihydro-4*H*-1-benzopyran-3,4-diol 32. White solid (0.32 g, 51%), mp 114–116 °C; $\delta_H(CDCl_3)$ 2.38 (1 H, d, *J* 6, OH), 2.58 (1 H, s, OH), 2.80 (2 H, s, 9-H), 3.88 (3 H, s, OCH_3), 3.7–4.1 (2 H, 2-H_2), 4.5 (1 H, d, *J* 6, 4-H), 5.20 (2 H, s, OBz) and 6.6–7.5 (12 H, m, arom); *m/z* 392 (M^+, 44%), 227 (17), 147 (7) and 137 (100).

3-(3-Benzyloxy-4-methoxybenzyl)-2,3-dihydro-4*H*-1-benzopyran-3,4-diol 33. Brown oil used without further purification (0.38 g, 85%); $\delta_H(CDCl_3)$ 2.68 (1 H, d, *J* 13, CH), 2.98 (1 H, d, *J* 13, CH), 3.2–4.4 (6 H, 2-H_2, 4-H, OCH_3), 4.6 (1 H, s, OH), 5.14 (2 H, 2 s, OBz), 5.20 (OH) and 6.8–7.5 (12 H, m, arom).

8-Benzyloxy-3-(3,4-dimethoxybenzyl)-7-methoxy-2,3-dihydro-4*H*-1-benzopyran-3,4-diol 34. Brown oil (0.48 g, 96%) (Found: C, 69.3; H, 6.35. $C_{26}H_{28}O_7$ requires C, 69.01; H, 6.24%); $\delta_H(CDCl_3)$ 2.75 (1 H, d, *J* 14, 9a-H), 3.0 (1 H, d, *J* 14, 9b-H), 3.5–4.2 (5 H), 3.85–3.9 (9 H, 3 OCH_3), 5.10 (2 H, s, OBz), 6.55 [1 H, d, *J* 8, 6(5)-H], 6.8–7.0 (4 H, m, arom) and 7.2–7.5 (5 H, m, arom); *m/z* 452 (M^+, 25%), 362 (4), 315 (3) and 151 (100).

7-Benzyloxy-3-(3,4-dimethoxybenzyl)-8-methoxy-2,3-dihydro-4*H*-1-benzopyran-3,4-diol 35. Flash chromatography with hexane–ethyl acetate (3:7) gave a white amorphous solid (0.28 g, 56%); $\delta_H(CDCl_3)$ 2.8 (1 H, d, *J* 16, CH), 3.05 (1 H, d, *J* 16, CH), 3.9 (3 × 3 H, s, OCH_3), 3.9–4.2 (3 H, m, 4-H and 2-H_2), 5.2 (2 H, s, OBz), 6.6 [1 H, d, *J* 9, 5(6)-H], 6.9 [1 H, d, *J* 9, 6(5)-H], 6.7–7.0 and 7.2–7.5 (8 H, m, arom).

Cyclization

The substrate was dissolved in acetic acid (10 cm^3 $mmol^{-1}$) and added with $HClO_4$ (70%; 1.2 cm^3 $mmol^{-1}$). The solution was stirred at room temperature for 3–24 h and then diluted with water, extracted with ethyl acetate and the extract dried and evaporated to dryness.

3,9,10-Trimethoxy-7,11b-dihydrobenz[*b*]indeno[1,2-*d*]pyran-4,6a-diol 6. Flash chromatography with hexane–ethyl acetate (1:1) and crystallization from toluene gave a white solid (0.2 g, 49%), mp 135–136 °C (Found: C, 66.0; H, 5.9. $C_{19}H_{20}O_6$ requires C, 66.27; H, 5.81%); $\delta_H(CDCl_3)$ 2.87 (1 H, d, *J* 16, 7a-H), 3.25 (1 H, d, *J* 16, 7b-H), 3.7–4.3 (12 H) and 6.5–7.0 (4 H, m, arom); *m/z* 344 (M^+, 100%), 325 (31) and 313 (5).

4,9,10-Trimethoxy-7,11b-dihydrobenz[*b*]indeno[1,2-*d*]pyran-3,6a-diol 7. Flash chromatography with hexane–ethyl acetate (1:1) gave 3-benzyloxy-4,9,10-trimethoxy-7,11b-dihydrobenz-[*b*]indeno[1,2-*d*]pyran-6a-ol **37** as a yellowish oil (0.6 g, 78%); $\delta_H(CDCl_3)$ 2.8 (1 H, d, *J* 16, 7a-H), 3.25 (1 H, d, *J* 16, 7b-H), 3.8–4.3 (12 H), 5.20 (2 H, s, OBz) and 6.6–7.6 (9 H, m, arom).

Catalytic hydrogenation of **37** with 10% Pd–C in ethanol and flash chromatography with hexane–ethyl acetate (1:1) gave compound **7** as a yellowish oil which was crystallized from ethyl ether–hexane (1:1) (20 mg, 16%), mp 85 °C (decomp.) (Found: C, 66.3; H, 5.9. $C_{19}H_{20}O_6$ requires C, 66.27; H, 5.85%); $\delta_H(CDCl_3)$ 2.85 (1 H, d, *J* 16, 7a-H), 3.27 (1 H, d, *J* 16, 7b-H), 3.85 (6 H, s, 2 OCH_3), 3.90 (3 H, s, OCH_3), 4.0–4.3 (3 H, 6-H_2 and 11b-H), 6.67 [1 H, d, *J* 9, 1(2)-H], 6.87 and 7.25 (2 × 1 H, s, 8-H and 11-H) and 7.02 [1 H, d, *J* 9, 2(1)-H].

9-Methoxy-7,11b-dihydrobenz[*b*]indeno[1,2-*d*]pyran-6a,10-diol 4. Chromatography with hexane–ethyl acetate (7:3) gave a white solid which was crystallized from toluene (126 mg, 54%), mp 142–143 °C (Found: C, 71.55; H, 5.9. $C_{17}H_{16}O_4$ requires C, 71.83; H, 5.63%); $\delta_H(CDCl_3)$ 2.75 (1 H, d, *J* 14, 7a-H), 3.32 (1 H, d, *J* 14, 7b-H), 3.7–4.2 (3 H, m, 2-H_2 and 11b-H), 5.52 (2 H, s, OBz), 6.7–7.5 (6 H, m, arom); *m/z* 284 (M^+, 100) and 265 (41%).

10-Methoxy-7,11b-dihydrobenz[*b*]indeno[1,2-*d*]pyran-6a,9-diol 5. The cyclization gave 10-benzyloxy-9-methoxy-7,11b-dihydrobenz[*b*]indeno[1,2-*d*]pyran-6a-ol **36** as a yellow oil (0.32 g, 88%); $\delta_H(CDCl_3)$ 2.8 (1 H, d, *J* 15, 7a-H), 3.24 (1 H, d, *J* 15, 7b-H), 3.8–4.2 (6 H), 5.1–5.2 (3 H, OH and OBz) and 6.78–7.5 (11 H, m, arom). Catalytic hydrogenation of **36** in ethanol with 10% Pd–C and purification on a silica gel column with hexane–ethyl acetate (1:1) gave **5** as a yellow oil which crystallized from ethyl acetate (0.24 g, 83%), mp 140 °C (Found: C, 71.4; H, 5.8. $C_{17}H_{16}O_4$ requires C, 71.83; H, 5.63%); $\delta_H(CDCl_3)$ 2.85 (1 H, d, *J* 15, 7a-H), 3.3 (1 H, d, *J* 15, 7b-H), 3.78–4.6 (4 H, m, 6-H_2 and 11b-H, OH), 3.80 (3 H, s, OCH_3), 5.55 (1 H, s, OH) and 6.8–7.5 (6 H, m, arom); *m/z* 284 (M^+, 100), 266 (43) and 137 (47%).

Synthesis of (−)-haematoxylin 1

3-(3,4-Dibenzyloxybenzylidene)-7,8-dihydroxy-2,3-dihydro-4*H*-1-benzopyran-4-one 23. The general procedure used for compounds **19**–**22** was followed. The crude reaction mixture was chromatographed with hexane–ethyl acetate (70:30) to give a mixture of the two *E*/*Z* isomers (TLC) as a yellow solid (4.38 g, 65%), mp 133–146 °C; $\delta_H(CDCl_3)$ 5.08–5.30 (6 H, OBz and 2-H), 5.5–6.0 (2 H, br, OH), 6.5–7.8 (16 H, m, arom and 9-H_2); *m/z* 389 (5%), 299 (3), 206 (8), 169 (4), 122 (30) and 91 (100).

7,8-Dihydroxy-3-(3′,4′-dihydroxybenzyl)-2,3-dihydro-4*H*-1-benzopyran-4-one 38. A mixture of **23** (2.5 g, 5.20 mmol), Pd–C (10%; 70 mg, 0.066 mmol), in absolute ethanol (15 cm^3) was stirred under H_2 for 16 h. The catalyst was filtered off and the filtrate evaporated to give a crude solid; treatment of this with hot diethyl ether gave a white solid (1.3 g, 83%), mp 208 °C; δ_H(DMSO) 2.6–2.85 (3 H, m, 9-H_2 and 3-H), 3.8–4.25 (2 H, m, 2-H_2), 6.25–7.25 (6 H, m, arom), 8.25–8.75 (3 H, br, OH), 9.89 (1 H, s, OH); *m/z* 302 (M^+, 88%), 180 (100), 152 (82) and 123 (98).

7,8-Dibenzyloxy-3-(3′,4′-dibenzyloxybenzyl)-2,3-dihydro-4*H*-1-benzopyran-4-one 39. To a mixture of **38** (5.54 g, 18.34 mmol), K_2CO_3 (20.23 g, 146 mmol) in butan-2-one (100 cm^3), benzyl bromide (13.85 cm^3, 81 mmol) was added with vigorous stirring. At the end of addition the reaction mixture was refluxed for 10 h. The inorganic solids were filtered off and the filtrate was evaporated. The crude product (10 g) was purified by chromatography with hexane–ethyl acetate (80:20) to give a yellow solid that crystallized with hexane–ethyl acetate to give a white solid (4.32 g, 36%), mp 112–113 °C (Found: C, 80.7; H, 5.8. $C_{44}H_{38}O_6$ requires C, 77.4; H, 5.78%); $\delta_H(CDCl_3)$ 2.3–3.2 (3 H, m, 9-H_2 and 3-H), 3.8–4.3 (2 H, m, 2-H_2), 5.0–5.2 (8 H, s, OBz) and 6.75–7.75 (25 H, m, arom); *m/z* 662 (M^+, 2%), 291 (26), 278 (36), 277 (98), 183 (32), 181 (100) and 163 (35).

(−)-3-Hydroxy-7,8-dibenzyloxy-3-(3′,4′-dibenzyloxy)-2,3-dihydro-4*H*-1-benzopyran-4-one 40. To a solution of Na-HMDS, generated *in situ* from HMDS (1.2 cm^3, 5.42 mmol) and NaH (0.16 g, 5.42 mmol) in THF (3 cm^3) cooled to −78 °C and stirred for 30 min, was added dropwise **39** (2.3 g, 3.47 mmol) dissolved in dry THF (18 cm^3). After the reaction mixture had been stirred at −78 °C for 30 min during which time it became yellow–orange, it was treated with (−)-8,8-dichlorocamphorylsulfonyloxaziridine (1.55 g, 5.21 mmol) in THF (6 cm^3). The mixture was stirred for 1 h and then quenched at −78 °C with saturated aq. $KHCO_3$ (15 cm^3) and water (40 cm^3) and extracted with ethyl acetate. The extract was worked up and the crude residue chromatographed with hexane–ethyl acetate (80:20) to give an oil; treatment of this with diethyl ether gave a white solid (0.98 g, 42%), mp 122–123 °C (Found: C, 77.1; H, 5.55. $C_{44}H_{38}O_7$ requires C, 77.86; H, 5.64%); $\delta_H(CDCl_3)$ 2.77 (2 H, s, 9-H), 3.5 (1 H, s, OH), 3.8–4.4 (2 H, AB, *J* 12, 2-H_2), 5.0–5.2 (8 H, s, OBz) and 6.5–7.5 (25 H, m, arom); *m/z* 678 (M^+, 1%), 181 (22) and 91 (100); $[\alpha]_D^{20}$ −25.0 (*c* 0.54, $CHCl_3$).

(−)-Haematoxylin tetrabenzyl ether 41. $NaBH_4$ (55 mg, 1.46 mmol) was added, in small portions, to a solution of **40** (0.40 g, 0.59 mmol) in absolute ethanol (4 cm^3) at 0 °C; the mixture was warmed to room temperature and stirred for 2 h and then treated with conc. HCl (1.1 cm^3). After being heated under reflux for 3 h the mixture was concentrated under reduced pressure, diluted with water (3 cm^3) and extracted with ethyl acetate. After work-up, chromatography with hexane–ethyl acetate gave a white solid which was recrystallized from hexane–ethyl acetate (0.24 g, 64%), mp 128–130 °C (Found: C, 77.5; H, 5.5. $C_{44}H_{38}O_6$ requires C, 79.74; H, 5.78%); $\delta_H(CDCl_3)$

2.7–3.25 (2 H, AB, *J* 16, 7-H), 3.60–4.25 (2 H, AB, *J* 11, 2-H_2), 4.08 (1 H, s, 11b-H), 5.0–5.2 (8 H, OBz) and 6.5–7.6 (24 H, m, arom); *m/z* 662 (M^+, 1%), 181 (8) and 91 (100); $[\alpha]_D^{20}$ −13.42 (*c* 0.514, $CHCl_3$). (+)-Haematoxylin tetrabenzyl ether, prepared from (+)-**1** by a literature method[8] showed $[\alpha]_D$ +12.97 (*c* 0.524, $CHCl_3$).

The enantiomeric excess was confirmed by a CLSR NMR experiment with tris(3-heptafluoropropylhydroxymethylcamphoryleuropium(III); the racemic mixture [prepared from equimolar amounts of (+)- and (−)-**41**] showed splitting of two AB systems with 10% of Eu complex added while (−)-**41** did not.

(−)-Haematoxylin 1. A solution of (−)-**41** (0.24 g, 0.36 mmol) in ethyl acetate was hydrogenated for 24 h with 10% Pd–C (30 mg, 0.024 mmol) as a catalyst. After removal of the catalyst the solution was evaporated under a N_2 atmosphere to give a pink solid which was purified by chromatography on polyamide (CC6, Macherey Nagel) eluting with MeOH to give an orange solid (22.3 mg, 20%) identical (TLC and ^{1}H NMR) to natural (+)-**1**; mp 152–154 °C [lit.,[1] 148–151 °C for (+)-**1**], $[\alpha]_D^{20}$ −100.8 (*c* 0.446, MeOH) {lit.,[2] $[\alpha]_D^{18}$ +90.3, *c* 1.04, MeOH for (+)-**1**}.

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