THE STRUCTURE OF YATEIN. DETERMINATION OF THE POSITIONS, AND CONFIGURATIONS OF BENZYL GROUPS IN LIGNANS OF THE 2,3-DIBENZYLBUTYROLACTONE TYPE*

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Dedicated to Professor Holger Erdtman on the occasion of his 80th birthday.

The structure of yatein isolated from *Libocedrus yateensis* has been determined as (2*R*,3*R*)-2-(3,4,5-trimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone (*Ia*). The assignment of individual signals of the ^{1}H NMR spectrum of yatein and further lignans of the 2,3-dibenzylbutyrolactone type is discussed and supported by the use of a lanthanide shift reagent and by mass spectrometry of deuterated derivatives *IIa*, *IIb*. A method of determination of absolute configuration of the centres $C_{(2)}$ and $C_{(3)}$ of these 2,3-dibenzylbutyrolactone lignans on the basis of a combination of information obtained from ^{1}H NMR spectra and optical rotation is proposed. The erroneous interpretation of the ORD and CD spectra used in some instances for the determination of absolute configuration of these substances, as well as the often repeated error in the assignment of benzyl and $C_{(2)}$—H, $C_{(3)}$—H protons in ^{1}H NMR spectra of *trans*-lignans is also discussed. A complete analysis of the ^{1}H NMR spectra permitted the proposition of the preferred conformation for *trans* and *cis* isomer *Ia* and *Ib*.

In the preceding paper[1] the components of the methanolic extract of the heartwood of *Libocedrus yateensis* were described and their use for chemosystematics within the genus *Cupressaceae* considered. One of the quantitatively most important compounds of the neutral fraction of this extract is yatein (*Ia*), a lignan of the 2,3-dibenzylbutanolide type. A description of the isolation, characteristic physical and spectral data and a structure proposal have already been published in a preliminary communication[1].

In the present paper stress is laid on the structure proof and the interpretation of the ^{1}H NMR spectra of yatein and its natural and synthetic stereoisomers and analogues. The possibility of the use of mass spectrometry for structural analysis of lignan lactones of this type is also described.

Yatein (*Ia*) was isolated chromatographically from the non-ketonic part of the methanolic extract using the procedure described in ref.[1]. From its infrared spectrum

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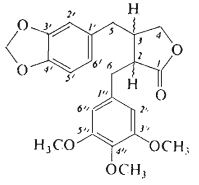
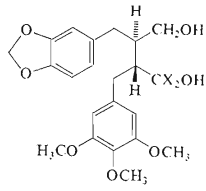
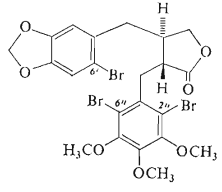
the presence of a five-membered lactone (1 244 and 1 764 cm^{-1}), an aromatic ring (1 490, 1 506 and 1 591 cm^{-1}) and a methylene dioxygroup (2 775 cm^{-1}) could be derived. On the basis of high-resolution mass spectrometry the molecular mass M^+ 400 and the composition $C_{22}H_{24}O_7$ could be derived as well as the masses of the fragments m/z 135 ($C_8H_7O_2$) and m/z 181 ($C_{10}H_{13}O_3$). Elemental analysis confirmed the elemental composition of the molecule and the content of three methoxy groups was also ascertained. From the composition of the fragments in the mass spectrum and from the mentioned analytical and spectral data it follows that the molecule contains one methylenedioxybenzyl group (m/z 135) and one trimethoxybenzyl group (m/z 181), both bound to the five-membered lactone which represents the rest of the molecule, $C_4H_4O_2$.



Ia; 2β H, 3α H
Ib; 2α H, 3α H
Ic; 2α H, 3β H



IIa; X = H
IIb; X = ^{2}H



III

The ^{1}H NMR spectrum (100 MHz) confirmed the presence of three aromatic methoxy groups, one methylenedioxy group and five aromatic hydrogens on three- or tetrasubstituted aromatic rings. This led to the confirmation of the fragments 3,4,5-trimethoxyphenyl and 3,4-methylenedioxyphenyl. The remaining fragment $C_6H_8O_2$ contains two hydrogens of the CH–O type, appearing as doublets of doublets at δ 4·18 or 3·88, another two hydrogens give a multiplet about δ 2·90, and the remaining four hydrogens an unsufficiently resolved multiplet at about δ 2·50. This part of the spectrum thus affords very little information on the structure.

In order to obtain a better dispersion of the signals we tried to make use of the solvent-effect, but none of the solvents used led to more distinct changes in the distribution of the mentioned signals. The measurement of the spectrum on a 360 MHz spectrometer (Fig. 1*b*) led to a slightly better resolution of the multiplets in the δ 2·90 and 2·50 ppm region, but in this case too its complete interpretation was not possible without further information. Therefore we made use of a shift reagent, the gradual addition of which gave 100 MHz spectra that permitted the assignment of hydrogens (by means of decoupling experiments) and also the extraction of the coupling constants in the fragment $C_6H_8O_2$ (Fig. 1*c*). From the dependence of the induced shifts on the molar ratio reagent : substrate, which is practically linear within the whole tested range (up to L : S about 1) except for protons of the trimethoxyphenyl ring (Fig. 2), the signals in the spectrum without the reagent were then assigned by extrapolation. The knowledge of the assignment permitted an almost complete analysis of the 360 MHz spectrum (for data see Table I and II). The ^{1}HNMR data obtained are consistent with the structure of 2,3-dibenzylbutyrolactone *I*.

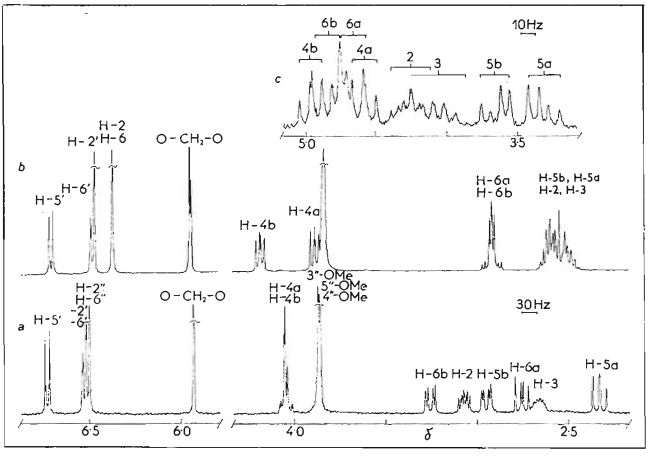


FIG. 1

The ^{1}HNMR spectra of isomers *Ia* and *Ib*. *a*) The spectrum of *cis*-dihydroanhydropodorhizol (*Ib*) on 360 MHz spectrometer, *b*) The spectrum of yatein (*Ia*) on 360 MHz spectrometer, *c*) The spectrum of yatein (*Ia*) after the use of the shift reagent on 100 MHz spectrometer

From the assignment of the hydrogens it follows that the four-proton multiplet at δ 2·50 does not contain the CH_2 hydrogens of both benzyl groups, as could be expected on the basis of the structural similarity of both fragments, and as was also erroneously interpreted by a number of authors, for example[2-7], but merely the CH_2 hydrogens of one benzyl group, together with two hydrogens of the butyrolactone ring ($C_{(2)}$—H and $C_{(3)}$—H), while CH_2 hydrogens of the second benzyl are shifted to δ 2·90 (Fig. 1*b*).

Since the benzylic CH_2 hydrogens do not interact with the hydrogens of the aromatic rings, the variously substituted benzyl groups cannot be localized unambiguously into positions 2 or 3. This determination of the mutual position of the benzyl groups was carried out by interpretation of the mass spectra of native yatein (*Ia*) and 2-deuterioyatein (*Id*), as well as of derivatives *IIa* and *IIb*.

When the structure of yatein was studied by mass spectrometry the electron impact fragmentation could be used as a basis, which was described for some similar

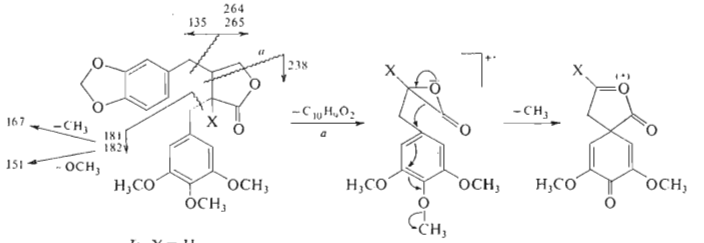
TABLE I

Chemical shifts of hydrogens in compounds *Ia*, *Ib*, *IIa* and *III*

Proton[a]	*Ia*	*Ib*	*IIa*	*III*
H-2	~2·57	3·07	1·88	3·03
H-3	~2·49	2·66		2·83
H-4a	3·88	4·04	3·54[b]	4·06
H-4b	4·18	4·06	3·82[b]	4·42
H-5a	~2·51	2·33	2·65[c]	2·50
H-5b	~2·62	2·95	2·75[c]	2·64
H-6a	2·88	2·76	2·64[c]	3·35
H-6b	2·94	3·25	2·79[c]	3·45
H-2′	6·46	6·50	6·64	6·47
H-5′	6·70	6·74	6·72	6·83
H-6′	6·47	6·53	6·60	—
OCH_2O	5·93; 5·94	5·92	5·92	5·96
H-2″, H-6″	6·36	6·50	6·36	—
3″-OCH_3, 5″-OCH_3	3·83	3·87	3·82	3·88
4″-OCH_2	3·83	3·86	3·83	3·94

[a] The spectra of compounds *Ia*, *Ib* were measured on a 360 MHz, of compounds *IIa*, *III* on a 200 MHz instrument, all in $CDCl_3$; [b] Hydrogens H-1a and H-1b are present in two-proton multiplets at 3·54 and 3·82 ppm; [c] the assignment of the pairs of benzyl protons may be interchanged; since H-2 and H-3 overlap, definite proof cannot be given.

substances[3,4,7,8]. Especially useful was the procedure described for the structural analysis of an analogous substance, pluviatolide[3]. For the molecular ion of yatein, M^+ 400, the elemental composition $C_{22}H_{24}O_7$ was found. The very abundant ions m/z 181, 182, 135 of its mass spectrum (Scheme 1) correspond to the trimethoxybenzyl and methylenedioxybenzyl ligands, bound to the central part of the molecule – a lactone ring of the composition $C_4H_4O_2$ (see also ions m/z 264 and 265, Scheme 1).



SCHEME 1

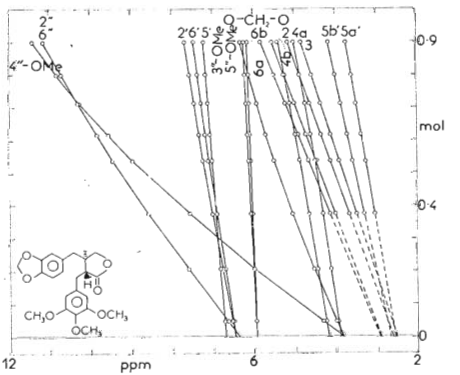


FIG. 2

The dependence of chemical shifts (δ) on the molar ratio reagent : substrate (L/S) for yatein (*Ia*) after the use of the shift reagent

For the determination of the relative position of the ligands in the molecule of yatein only those peaks of the ions are utilizable which are formed by the cleavage of the lactone ring. On cleavage *a* (Scheme 1) of the M^+ of yatein the ion *m/z* 238 is formed, with the composition $C_{12}H_{14}O_5$, which is expected for the fragment with the trimethoxybenzyl group. The ion *m/z* 161 is the result of the same cleavage if it takes place with the retention of the charge on the second part of M^+, containing a methylenedioxybenzyl group. In this case the cleavage takes place with a hydrogen transfer (Scheme 2), while, for example, in the mass spectrum of pluviatolide[3] the ion 162 was observed instead of the ion 161, the former being a result of a simple cleavage of the lactone ring. The elemental composition of the ion 161, $C_{10}H_9O_3$, which is in agreement with the presented interpretation, determines together with the interpretation of ion 238 the position of both ligands in the molecule of yatein unambiguously, as shown by formula *I*. In agreement with this conclusion the ion *m/z* 238 in the mass spectrum of 2-deuterioyatein (*Id*) prepared for the confirmation of the fragmentation schemes is shifted to *m/z* 239, while no shift took place in the case

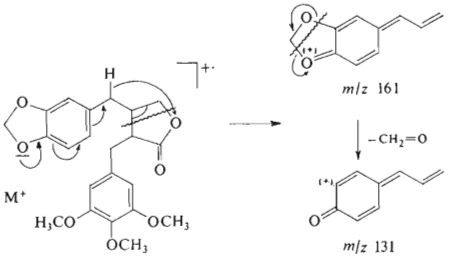
TABLE II

Coupling constants of hydrogens in compounds *Ia*, *Ib*, *IIa* and *III*

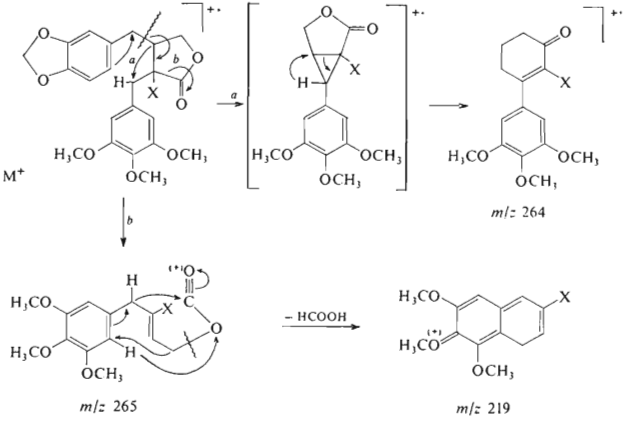
Values	*Ia*[a]	*Ia*[b,c]	*Ib*[a,c]	*Ib*[b]	*IIa*[d,e]	*III*[e]
$J_{3,2}$	[f]	8·5	7·0	7·0	[f]	5·4
$J_{2,6a}$	~6·0	5·5	10·4	10·0	6·8	10·4
$J_{2,6b}$	~5·0	4·5	4·7	4·0	3·4	6·2
$J_{3,4a}$	~7·5	8·0	5·2	[g]	4·4	4·9
$J_{3,4b}$	~7·0	7·5	1·6	[g]	2·2	6·9
$J_{3,5a}$	~8·5	7·5	12·2	12·0	6·8	8·2
$J_{3,5b}$	~6·5	7·0	4·0	3·5	3·4	7·4
$J_{4a,4b}$	−9·1	−9·3	− 9·4	[f]	−11·3	− 9·5
$J_{5a,5b}$	−13·5	−13·8	−13·8	−13·5	−13·6	−13·7
$J_{6a,6b}$	−14·0	−14·0	−14·9	−14·5	−13·8	−14·0
$J_{2',5'}$	0·4	0·5	0·5	[f]	0	0
$J_{2',6'}$	1·6	1·5	1·4	[f]	1·6	—
$J_{5',6'}$	7·7	7·5	7·0	[f]	7·8	—

[a] Values obtained from the 360 MHz spectrum; [b] values obtained from the 100 MHz spectra in $CDCl_3$ after addition of the shift reagent (molar ratio L : S = 0·5); [c] the values are corrected using the simulation procedure (standard version, on Varian XL-200) up to an optimum agreement with the experimental spectrum; [d] further values: $J_{1a,1b} = -11·3$, $J_{1a,2} = 4·4$ and $J_{1b,2} = 2·2$ Hz; [e] values obtained from the 200 MHz spectra; [f] the *J* values could not be obtained; [g] only the sum $J_{3,4a} + J_{3,4b} = 6·5$ Hz was determined.

of the peak m/z 161. The peak m/z 264 in the spectrum of compound *Id* is shifted to m/z 265. From this it follows that the splitting off of the ligand from $C_{(3)}$ does not take place as 1,2-elimination, under formation of a double bond conjugated with the

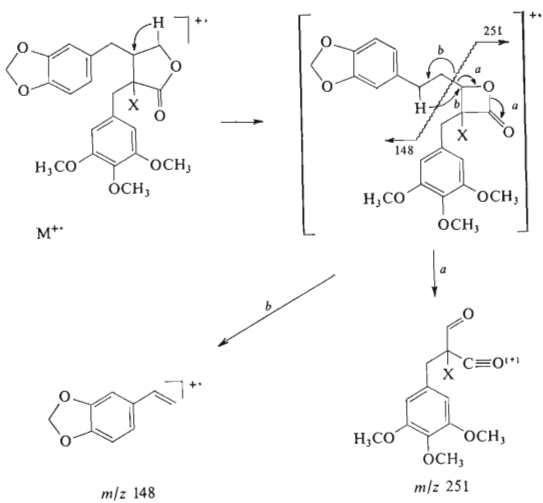


SCHEME 2



SCHEME 3

C=O group, as assumed earlier[3], but rather as 1,3-elimination (Scheme 3). The ion 265, formed by the loss of the ligand from $C_{(3)}$ without a hydrogen transfer, is less stable and it decomposes under elimination of the particle HCOOH and the formation of ion m/z 219, as shown in Scheme 3. On the other hand, the elimination of the second ligand with a hydrogen transfer, leading to the formation of ion m/z 182, evidently takes place as 1,2-elimination. This follows from comparison with the mass spectrum of the *cis*-isomer *Ib*, which is identical with the spectrum of yatein, except for the fact that the ion m/z 182 is practically absent in it. The suppression of the mentioned elimination is evidently caused by the excessively large distance of the atom H on $C_{(3)}$ in the position *trans* to trimethoxybenzyl, which should be transferred to the eliminated group.



SCHEME 4

For the explanation of the formation of the fragments m/z 148 and 251 it had to be assumed that in M^+ of yatein a contraction of the lactone ring might take place and that the rearranged intermediate immediately decomposes according to Scheme 4. In accordance with this the peak 251 in the spectrum of 2-deuterioyatein (*Id*) is shifted by 1 mass unit, the same as the peaks m/z 219, 223, 238, 264 and 265. (X = H for

yatein (*I*); X = ^2H for 2-deuterioyatein (*Id*) in Schemes 1, 3 and 4). The elemental composition determined by high resolution measurement of the masses of all mentioned ions also agrees with the proposed fragmentation schemes 1–4 and with the structure proposed for yatein.

Since the relative position of both ligands on the lactone ring could be determined directly from the mass spectrum of the native substance on the basis of the interpretation of only three relatively low peaks, the interpretation of the mass spectra of diols *IIa* and *IIb*, prepared for this purpose by reduction of yatein with $LiAlH_4$ or $LiAl^2H_4$, were also made use of for the confirmation of the structure. The cleavage of the $C_{(2)}$—$C_{(3)}$ bond in diol *IIa* under electron impact gives rise to a fragment of *m/z* 225 (Table III) for which the composition $C_{12}H_{17}O_4$ was found on the basis of high resolution data, expected for the fragment containing the trimethoxybenzyl group. The shift of the ion *m/z* 225 in the mass spectrum of the deuterated derivative *IIb* to *m/z* 227 indicates that the carbinol group of this ion was formed on reduction of the lactone carbonyl group and confirms thus the location of the substituents as in formula *I*. Some more abundant fragments in the mass spectrum of diols *IIa* and *IIb* are given in Table III. The mentioned procedure is simple and unambiguous

TABLE III

Comparison of the fragmentation of diols *IIa* and *IIb*

Main fragments		Diol *IIa* (*m/z*)	Diol *IIb* (*m/z*)
Composition[a]	structure[b]		
$C_{22}H_{28}O_7$	M^+	404	406
$C_{14}H_{17}O_3$	$CH_2{=}\overset{(+)}{C}{-}\overset{CX_2}{\overset{\|\|}{C}}{-}CH_2{-}R^1$	233	235
$C_{14}H_{18}O_4$		250	252
$C_{12}H_{17}O_4$	$HO{-}CX_2{-}\dot{C}H{-}CH_2{-}R^1$	225	227
$C_{10}H_{13}O_3$	$\overset{(+)}{C}H_2{-}R^1$	181	181
$C_{10}H_{14}O_3$	$CH_2X{-}R^{1c}$	182	183
$C_8H_7O_2$	$\overset{(+)}{C}H_2{-}R^2$	135	135

[a] Composition obtained by high resolution measurement of the diol *IIa*; [b] X = H for the fragments of *IIa* and X = ^2H for the fragments of *IIb*; [c] transfer of H and ^2H respectively (1/2 : : 1/2); R^1: 3,4,5-trimethoxyphenyl. R^2: 3,4-methylenedioxyphenyl.

and it may be used for the determination of the mutual position of the benzyl groups of lignan lactones with non-equivalent benzyl groups.

For the determination of the relative configuration of $C_{(2)}$ and $C_{(3)}$ the value of the vicinal coupling $J_{2,3} = 8{\cdot}5$ Hz obtained from the spectrum of yatein after the addition of the shift reagent cannot be used. In a five-membered cycle this value may correspond – in principle – both to the *cis* and *trans* derivative. Substance I, with the configurations 2αH, 3βH (hence *Ic*) has already been described[4]. However, Japanese authors[5] later corrected the structure of this substance to 2βH, 3αH (*i.e.* to *Ia*). The only comparable physical constant, the value of optical rotation, was evidence for the identity of yatein ($[\alpha]_D^{20}$ −28·4°, $CHCl_3$) with a substance isolated from *Bursera schlechtendalii* ($[\alpha]_D^{23}$ −29·8°, $CHCl_3$)[3]. For an unambiguous determination of the configuration of $C_{(2)}$ and $C_{(3)}$ in yatein we carried out chemical correlation with *cis*-dihydroanhydropodorhizol[9] (*Ib*). When epimerizing substance *Ib* ($[\alpha]_D^{21}$ +71·2°, $CHCl_3$) (ref.[9]) in alkaline medium we obtained a product which according to its infrared, mass, ^{1}H NMR and CD spectrum was identical with yatein. Optical rotation of epimerized dihydroanhydropodorhizol ($[\alpha]_D^{20}$ −25·5°, *c* 0·35, $CHCl_3$) was slightly lower than that of yatein which may be due to the presence of a small amount of non-epimerized *cis*-isomer (undetected in ^{1}H NMR spectrum) which has the same R_F values as the *trans*-isomer in various solvent systems. On the basis of the above-described correlation yatein was assigned the structure of (2*R*,3*R*)-2-(3,4,5-trimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone (*Ia*).

TABLE IV

Induced chemical shifts of protons in isomers *Ia* and *Ib* after addition of the shift reagent (mol. ratio L : S = 0·5)

Proton	*Ia*	*Ib*	Proton	*Ia*	*Ib*
H-2	1·43	1·35	H-2′	0·72	0·42
H-3	1·32	0·80	H-5′	0·30	0·08
H-4a	0·58	0·53	H-6′	0·59	0·36
H-4b	0·63	0·49	OCH_2O	0·11; 0·16	0·03
H-5a	0·68	0·76	H-2″	2·88	2·60
H-5b	0·88	0·94	H-6″		
H-6a	1·49	1·44	3″-OCH_3	1·55	1·43
H-6b	1·58	1·58	5″-OCH_3		
			4″-OCH_3	4·81	4·70

The ^{1}H NMR spectra of *Ia* and *Ib* are different (Fig. 1). Unlike the poorly interpretable spectrum of substance *Ia* (Fig. 1*b*) there are in the 360 MHz spectrum of substance *Ib* (Fig. 1*a*) all eight non-aromatic hydrogens well resolved. The reasons must lie in the different *trans* or *cis* configuration of the benzyl substituents in *Ia* or *Ib* and in the conformations of the molecules of both substances induced by it. In order to compare the behaviour of substances *Ia* and *Ib* we also measured the spectrum of substance *Ib* in the presence of a shift reagent in an analogous manner. The results of the measurement are summarized in Fig. 3 and the induced shifts $\Delta\delta$ in Table IV. The observed $\Delta\delta$ values (for L : S = 0·5) of corresponding protons in both substances are very similar (differences $\Delta\delta < 0{\cdot}15$ ppm), with the exception of aromatic hydrogens (difference $\Delta\delta$ 0·2 to 0·3 ppm) and $C_{(3)}$—H (difference $\Delta\delta \approx$ $\approx$ 0·5 ppm). The most distinct $\Delta\delta$ values are shown by $C_{(3')}$—OCH_3, which indicates that the shift reagent forms stronger complexes with the ether oxygens of the methoxy groups than with the lactone group. From a comparison of the values of the available coupling constants in $CDCl_3$ and after addition of the shift reagent (difference $\leqq$1 Hz) it follows that complexation does not lead to more pronounced conformational changes of molecules *Ia* and *Ib*. Hence, vicinal coupling constants and their known dependence on the dihedral angle were used for conformational analysis together with an analysis of models.

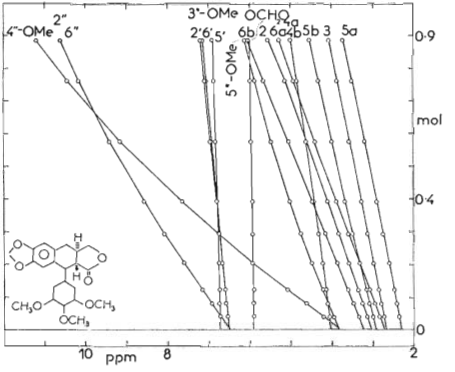


FIG. 3

The dependence of chemical shifts (δ) on the molar ratio reagent : substrate (L/S) for *cis*-dihydroanhydropodorhizol (*Ib*) after the use of the shift reagent

Spatial Arrangement of Lignans

Information on the conformation of the five-membered lactone ring is included in the ^3J values of the fragment $—C_{(2)}H—C_{(3)}H—C_{(4)}H_2—$. In *cis*-derivative *Ib* the values $J_{3,4a} = 5{\cdot}2$ and $J_{3,4b} = 1{\cdot}6$ Hz indicate conformation *A* (this and all subsequent conformations discussed are surveyed in Fig. 4) around the $C_{(3)}—C_{(4)}$ bond with the hydrogen $C_{(3)}—H$ directed inside the $H—C_{(4)}—H$ angle and with the dihedral angles about 40° and 80°. The value of $J_{2,3} \approx 7$ Hz is in agreement with the *cis* arrangement of the hydrogens on $C_{(2)}$ and $C_{(3)}$ when the dihedral angle is about 30° (see *B*). Therefore, the butyrolactone ring should assume conformation *C*, with the $C_{(3)}$ carbon atom under an approximately planar arrangement of the atoms $C_{(2)}—C_{(1)}—O—C_{(4)}$.

trans-Derivative *Ia* has both coupling constants $J_{3,4}$ distinctly higher ($J_{3,4a} = 8{\cdot}0$, $J_{3,4b} = 7{\cdot}5$ Hz). The hydrogen $C_{(3)}—H$ also must be directed outside the region of the angle $H—C_{(4)}—H$, as shown by conformation *D*, with dihedral angles $C_{(3)}H—C_{(4)}H$ about 20° and 140°. Together with the value $J_{2,3} \approx 8{\cdot}5$ Hz which, when in *trans* arrangement, corresponds to the dihedral angle of hydrogens about 150°C (see *E*), this indicates conformation *F* for butyrolactone, which again has an approximately planar arrangement of the atoms $C_{(2)}—C_{(1)}—O—C_{(4)}$, but with the $C_{(3)}$ carbon atom above this plane.

The orientation of the benzyl substituents with respect to the butyrolactone ring is given by the conformation around the bonds $C_{(3)}—C_{(5)}$ and $C_{(2)}—C_{(6)}$ and it thus follows from the values $J_{3,5}$ and $J_{2,6}$. If only the staggered rotamers are considered as probable, then for the *cis*-isomer *Ib* it follows from the observed values $J_{3,5a} = 12{\cdot}2$, $J_{3,5b} = 4{\cdot}0$, or $J_{2,6a} = 10{\cdot}4$ and $J_{2,6b} = 4{\cdot}7$ Hz, that one of the rotamers G_2, G_3 or H_2, H_3 is distinctly preferred, and that the forms G_1 or H_1 are very poorly populated. A more distinct representation of the forms G_1 and H_1 is also excluded by large steric interactions of the benzyl substituents oriented in this manner. A consideration of the steric interactions further leads to the preference of the form G_3 as against G_2, or H_3 as against H_2. This is in agreement with the observation that the CH_2 hydrogens on each of the two benzyl groups are distinctly non-equivalent (difference of shifts 0·62 ppm for $C_{(5)}H_2$ and 0·49 ppm for $C_{(6)}H_2$), evidently in consequence of their different orientation with respect to the lactone ring, as is the case in rotamers G_3 and H_3, and that the hydrogen atoms for which an upfield shift may be expected, due to the shielding effect of the carbonyl group (directed above the lactone ring), also have a large ^3J constant (Table II). If this assignment is accepted, then the representation of the rotamers $G_1 : G_2 : G_3 = 0 : 13 : 87$ around the $C_{(3)}—C_{(5)}$ bond and $H_1 : H_2 : H_3 = 10 : 19 : 71$ around the $C_{(2)}—C_{(6)}$ bond may be determined approximately from the values $^3J_{trans} = 13{\cdot}6$ Hz and $^3J_{gauche} = 2{\cdot}6$ Hz (ref.[10]) and from the experimental values for $J_{3,5}$ and $J_{2,6}$. Hence, the *cis*-derivative *Ib* should exist predominantly in the form *J* with the conformation *C* of the butyrolactone ring.

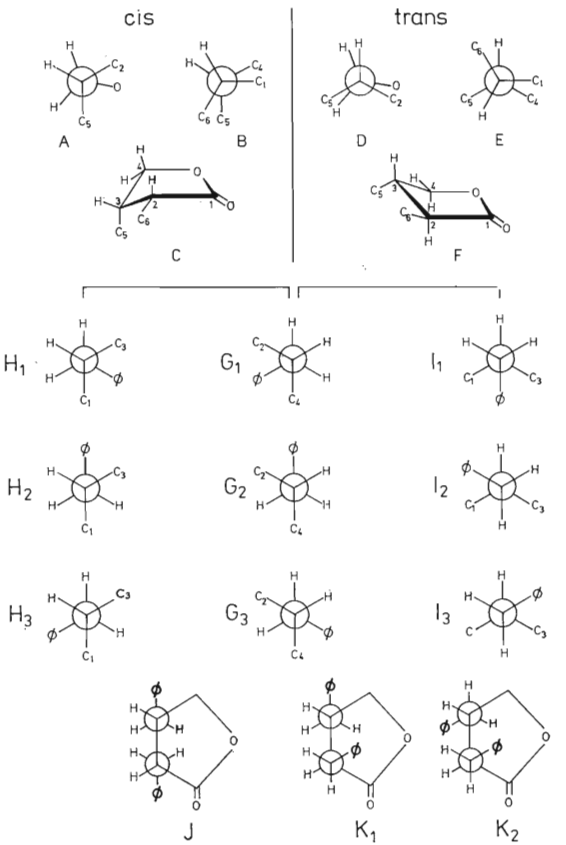


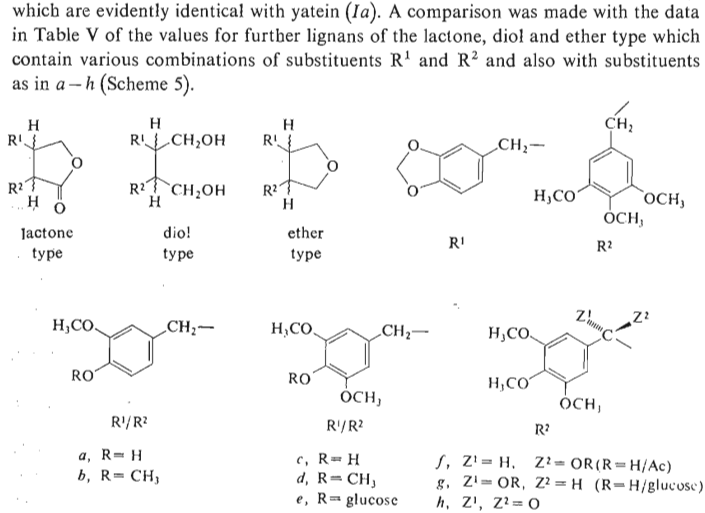
FIG. 4

The spatial arrangement of isomers *Ia* and *Ib*. A survey of conformations discussed in the text

In *trans*-derivative *Ia* is the situation considerably different. The non-equivalence of the hydrogens in each of the two benzyl groups is very little (the difference in shifts is 0·11 ppm for $C_{(5)}H_2$ and 0·06 ppm for $C_{(6)}H_2$) and the vicinal interactions also have similar values ($J_{3,5a} = 7{\cdot}5$, $J_{3,5b} \approx 7{\cdot}0$ and $J_{2,6a} \approx 5{\cdot}5$, $J_{2,6b} \approx 4{\cdot}5$ Hz). Both facts may be explained by the substantially higher mobility of the benzyl groups, evidently in consequence of the disappearance of steric interactions in the *trans*-arrangement, which leads to a comparable proportion of the rotamers around the $C_{(3)}$—$C_{(5)}$ and $C_{(2)}$—$C_{(6)}$ bonds. Analogously as in *cis*-derivative the proportion of the rotamers $G_1 : G_2 : G_3 = 16 : 45 : 40$ for the $C_{(3)}$—$C_{(5)}$ and $I_1 : I_2 : I_3 = 57 : 26 : 17$ for the $C_{(2)}$—$C_{(6)}$ bond can be calculated approximately for the *trans*-derivative. The values for G_2 and G_3 or I_2 and I_3 may be interchanged (the hydrogen atoms cannot be assigned). Therefore the *trans*-derivative *Ia* can exist in the preferred forms K_1 and K_2 with the conformation *F* of the butyrolactone ring. A comparison of our ^{1}H NMR spectra of derivatives *Ia* and *Ib* with the published NMR data of other *trans*- or *cis*-2,3-dibenzylbutyrolactone lignans[2-7,29] shows that the spectra of both types of isomers have distinctly characteristic features, following evidently from the above discussed conformation differences which can be utilized for the determination of relative configurations on $C_{(2)}$ and $C_{(3)}$. In *cis*-derivatives the benzylic CH_2 and $C_{(2)}$—H, $C_{(3)}$—H are relatively well resolved within a broad range (δ 2·3–3·3), while the hydrogens in each of the benzyl groups are distinctly non-equivalent, though the hydrogens of the $C_{(4)}$-methylene group are almost equivalent in the δ 4·0–4·1 range (Fig. 1*a*). On the other hand, for *trans*-derivatives a poorly resolved spectrum with a four-proton multiplet (H–2, H–3, H–5a, H–5b) at δ 2·5–2·6, a two-proton multiplet (H–6a, H–6b) at $\delta \approx 2{\cdot}9$, a very small non-equivalence of the protons of each of the two benzyl groups and distinctly non-equivalent hydrogens of the $C_{(4)}$-methylene group ($\delta \approx 3{\cdot}9$ and 4·2) is typical (Fig. 1*b*).

Hence, using ^{1}H NMR spectroscopy it may be determined whether the relative configuration of the 2,3-dibenzylbutyrolactone lignan is *cis* or *trans*, but it cannot be determined which of the two possible *cis* or *trans* configurations is present. For this determination the correlation with a stereochemically unambiguously assigned lignan is indispensable. Recently, stereoselective total syntheses of antileukemia lactones[11-13], diols[12,14] and ethers[14] have been described, containing the 3,4-methylenedioxybenzyl and 3,4,5-trimethoxybenzyl group in positions $C_{(2)}$ and $C_{(3)}$. Their molar optical rotations (Table V) lend themselves for correlations of their structures by means of optical rotation measurements. Yatein (*Ia*), with a molar rotation of $[\Phi]_D$ −114° and diol *IIa* with $[\Phi]_D$ −125°, formed by its reduction with lithium aluminum hydride, fall well within the 2βH, 3αH group. To this group belongs also the lignan which was isolated from *Bursera schlechtendalii* with $[\Phi]_D$ −119° (according to ref.[4]), as well as the derivative prepared by epimerization of *cis*-dihydroanhydropodorhisol with $[\Phi]_D$ −102° and $[\Phi]_D$ −86° (according to ref.[5])

and the synthetic (−)-deoxypodorhisone with $[\Phi]_D$ −89° (according to ref.[13]), which are evidently identical with yatein (*Ia*). A comparison was made with the data in Table V of the values for further lignans of the lactone, diol and ether type which contain various combinations of substituents R^1 and R^2 and also with substituents as in *a* – *h* (Scheme 5).



SCHEME 5

Literature data for known lignans were compared with the data in Table V. According to this comparison all natural lignans belong to the 2βH, 3αH series. The predominant part of them is of a lactone type[3–7,9,15–20]; some of them, as for example the diesters of the lignan from *Salvia plebeia*[21] are of the diol type, or burseran from *Bursera microphilla*[22] is of the ether type. The last assignment is merely indirect, on the basis of the comparison of the properties of natural burseran[22] with the stereoselectively synthetized burserans[14]. Thujaplicatin[2] and its methyl ethers with $[\Phi]_D$ −156° or $[\Phi]_D$ −189° must belong to the 2βH, 3αH series even though the original paper implies the 2αH, 3βH configuration. A correlation, carried out by a synthesis from a racemic synthon proves only the relative configuration *trans*, but admits both its possibilities, because the comparison was carried out on the basis of the IR and ^{1}H NMR spectra only. Optical rotations and correlations of their structural analogues[9,15] classify thujaplicatin and its derivatives among 2βH, 3αH derivatives. The lignans of the remaining configurations were prepared either synthetically[11–14] or by conversion of natural lignans[9,15–17]. In the 2βH, 3βH series no lignan is known so far.

In lignans of lactone type with $R^2 = f$ or g a further chirality centre has been introduced. The measured values of specific rotation and the structures described[9,12,23] agree well even in this case with the assignment of the 2βH, 3αH configuration. However, lignans of the lactone type with $R^2 = h$ are an exception, *i.e.* lignans with a further carbonyl group forming a new inherently chiral system in the molecule. Thus, for podorhizone with $[\Phi]_D$ +329° (according to ref.[9]) the structure with the configuration 2βH, 3αH and for the stereoselectively synthetized (−)-podorhizone with $[\Phi]_D$ −166° (according to ref.[11]) the configuration 2αH, 3βH was assigned, for which the results given in Table V no longer apply.

Some authors[3,5,17] carried out assignments of absolute configuration by means of ORD or CD spectra. Their assignment follows from the interpretation of Cotton effects at $\lambda_1 \approx 280$ and at $\lambda_2 \approx 240$ nm. According to us the last Cotton effect is due to the chirality of the benzyl groups with hindered rotation. This is also supported by the CD spectrum of diol *IIa*, or (−)-cubein[17] (which contains a hydroxy group instead the lactone carbonyl), but the character of their CD spectra in the mentioned region is very similar to that of butyrolactone lignans. The Cotton effect of the five-membered lactone carbonyl appears in the region of about $\lambda \approx 215$ nm (ref.[24,25]), which is in agreement with the maximum at $\lambda_3 = 214$ nm in the UV spectrum of yatein. In this region the $\Delta\varepsilon$ value is not reliable, however, because the contribution of a very high extinction of the aromatic rings is apparent. For these reasons the possibility of using ORD and CD spectra is limited merely to the assignment of relative configuration *cis* or *trans* for lignan lactones of this type. However, so few such assignments have been done that it is impossible to use them as in the case of optical rotations.

From our study of the structure of yatein and its structural analogues it follows that from the combination of the information from the ^{1}H NMR spectrum and the

TABLE V

Molar optical rotations (literature data[9,12–14], in chloroform, 20–25°C) of lignans of various configurations on $C_{(2)}$ and $C_{(3)}$

Type of lignan[a]	*cis*		*trans*	
	2αH, 3αH	2βH, 3βH	2αH, 3βH	2βH, 3αH
Lactone-type	+285°	[b]	+ 85°	− 89°
Diol-type	+ 37°	[b]	+122°	−115°
Ether-type	+ 22°	[b]	+152°	−141°

[a] The types of lignans are shown in Scheme 5; [b] not known so far.

optical rotation values the absolute configuration of the centres $C_{(2)}$ and $C_{(3)}$ in the lignans of the 2,3-dibenzylbutanolide type can be determined better and more reliably.

For a better characterization of yatein (*Ia*) which was not obtained in crystalline form by other authors[4,5,13] either, its tribromo derivative *III* with m.p. 183–184°C and $[\Phi]_D$ −25° was prepared. The ^{1}H NMR data are given in Tables I and II. The distribution of bromine atoms into position 6′,2″,6″ follows from the fragmentation in the mass spectrum (m/z = 213 and 215 for CH_2O_2—C_6H_2Br—CH_2— and m/z = 337, 339 and 341 for $(CH_3O)_3$—C_6Br_2—CH_2—) and from the ^{1}H NMR spectrum, where the methoxyl signals (δ 3·88, 6 H and 3·94, 3 H) require a symmetrical distribution of bromine on trimethoxybenzyl substituent into positions 2″,6″ and the signals of the two non-equivalent aromatic hydrogens (δ 6·47, 1 H and 6·38, 1 H) require their *para*-arrangement and hence the substitution of bromine into position 6′.

The biological activity[4,11,26] of some lignans of 2,3-dibenzylbutyrolactone type and their assumed biological importance not only in plants but in animal and human bodies as well[27–30] increased the interest in these compounds. The mentioned findings will facilitate the determinations of the structures of lignans of this type, mainly with respect to the determination of absolute configuration of benzyl groups and the determination of conformations of their molecules in solution. This is obviously important for the study of the relationship between the structure and biological activity of the whole group of lignan lactones.

EXPERIMENTAL

^{1}H NMR spectra of compounds *Ia*, *Ib* were measured on a Bruker WH-360 (360 MHz) spectrometer and compounds *IIa* and *III* on a Varian XL-200 (200 MHz) instrument in the FT mode in $CDCl_3$, using tetramethylsilane as internal standard. The data obtained are in Tables I and II. The spectra of compounds *Ia* and *Ib* in the presence of the shift reagent were measured on a Varian HA-100 (100 MHz) instrument, in CW mode, in the following manner: 18·9 mg of compound *Ia* (or 13·6 mg of compound *Ib*) were dissolved in 0·5 ml of $CDCl_3$ (dried over molecular sieve Nalcit 4) and weighed amounts of freshly sublimated tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,5-octanedione) europium(*III*) (Merck) were gradually added. This reagent will be indicated in the further text as $Eu(fod)_3$. The dependence of the chemical shifts of hydrogen on the molar ratio shift reagent : substrate (L : S) is shown in Fig. 2 and 3. The values of induced hydrogen shifts (Δδ) at a molar ratio L : S = 0·5 are given in Table IV and the coupling constants obtained in Table II. The ^{13}C NMR spectrum of compound *Ia* was measured on a FT NMR spectrometer Jeol FX-100 (at 25·15 MHz) in $CDCl_3$, with tetramethylsilane as internal standard, using a broad band decoupling of hydrogens. The assignment of the signals was carried out on the basis of a comparison with the described assigned spectra of similar compounds[20].

The mass spectra were measured on a double focussing mass spectrometer AEI MS-902. The samples were introduced by direct inlet probe into ionic source at 140–160°C. The low resolution mass spectra were recorded by the resolving power $m_1/(m_1 - m_2) = 10\,000$. The accurate masses

were found in the ±3 ppm range of the calculated value. Partial mass spectra of *Ia*, *IIa* and *III* are given below. The masses followed (in brackets) by elemental compositions calculated from accurate masses found, and the relative abundance in percent of base peak, are also given.

Optical rotations were measured on PE 141 MC polarimeter, CD spectra on a Roussel–Jouan Dichrographe CD-185, IR spectra on a C. Zeiss UR-20 spectrometer and UV spectra on a CF-4 Optica Milano instrument. The melting points were determined on a Kofler block and they are not corrected. Chromatographic separations were carried out on Kieselgel (Merck) and on Silpearl (Kavalier, Votice), TLC on Kieselgel GF 254 (Merck).

Yatein (*Ia*)

Isolation of yatein (*Ia*) from *Libocedrus yateensis* is described in the preceding paper[1]. It was obtained in the form of a glassy mass, $[\alpha]_D^{20}$ −28·4° (c = 0·32, $CHCl_3$). IR spectrum ($CHCl_3$): 1 764, 1 244, 1 591, 1 506, 1 490 and 2 775 cm^{-1}. UV spectrum (ethanol): λ_{max_1} 288 nm (log ε 3·56), λ_{max_2} 258 nm (log ε 2·87) and λ_{max_3} 214 nm (log ε 4·31). CD spectrum (methanol): $\Delta\varepsilon_{277}$ −0·56, $\Delta\varepsilon_{254}$ −0·15, $\Delta\varepsilon_{238}$ −2·12. ^{1}H NMR spectrum is shown in Tables I and II, ^{13}C NMR spectrum: $C_{(1)}$ 178·5, $C_{(2)}$ 46·4, $C_{(3)}$ 41·0, $C_{(4)}$ 71·1, $C_{(5)}$ 38·3, $C_{(6)}$ 35·2, $C_{(1')}$ 131·6, $C_{(2')}$ 108·8, $C_{(3')}$ 147·9, $C_{(4')}$ 146·4, $C_{(5')}$ 108·2, $C_{(6')}$ 121·5, OCH_2O 101·0, $C_{(1'')}$ 133·3, $C_{(2'')}$ 106·2, $C_{(3'')}$ 153·2, $C_{(4'')}$ 136·8, $C_{(5'')}$ 153·2, $C_{(6'')}$ 106·2, $C_{(3'')}$ —OCH_3 65·1, $C_{(4'')}$ —OCH_3 60·8, $C_{(5'')}$ —OCH_3 56·1. Mass spectrum: 77 (22·1), 79 (10·4), 91 (8·7), 105 (9·4), 131 (C_9H_7O, 10), 135 ($C_8H_7O_2$, 45·8), 136 (15·8), 148 (10·6), 151 (12·5), 161 ($C_{10}H_9O_2$, 8·3), 167 (10·8), 181 ($C_{10}H_{13}O_3$, 100), 182 ($C_{10}H_{14}O_3$, 62·5), 219 ($C_{13}H_{15}O_3$, 6·7), 223 ($C_{11}H_{11}O_5$, 2·7), 238 ($C_{12}H_{14}O_5$, 2·6), 251 ($C_{13}H_{15}O_5$, 5·8), 264 ($C_{14}H_{16}O_5$, 11·2), 265 (4·2), 385 (1·9), 400 (M^+, $C_{22}H_{24}O_7$, 79).

Epimerization of *cis*-Dihydroanhydropodorhizol (*Ib*)

A solution of *Ib* (25 mg) in 3% methanolic potassium hydroxide (5 ml) was allowed to stand at room temperature for 75 h, then acidified with acetic acid (1 ml) and evaporated under reduced pressure at 60°C. The residue was treated with water (10 ml) and 1M-H_2SO_4 (3 ml) and the solution extracted with ether. The ethereal extract was neutralized with a saturated sodium hydrogen carbonate solution, washed with water, dried over sodium sulfate and evaporated. The residue was filtered on a short silica gel column using chloroform. The product obtained (20 mg) had $[\alpha]_D^{20}$ −25·5° (c = 0·35, $CHCl_3$) and had identical IR, ^{1}H NMR, CD and mass spectrum with yatein (*Ia*).

2-Deuterioyatein (*Id*)

A solution of yatein (*Ia*, 70 mg) in dioxane (20 ml) was added dropwise and under nitrogen to a stirred solution of sodium (80 mg) in a mixture of ^2H_2O (99·5%, 10 ml) and dioxane (20 ml). After addition of the whole amount the mixture was refluxed for 30 min, evaporated and dioxane (20 ml) and ^2H_2O (10 ml) were added again. This solution was refluxed for another 20 min, acidified with a 50% solution of H_2SO_4 in ^2H_2O and extracted with ether. The extract was dried, evaporated and purified on a short column of silica gel deactivated with 15% of ^2H_2O, using light petroleum–dichloromethane for elution. The product was used for mass spectrometry.

Diol *IIa*

Yatein (*Ia*, 360 mg) in ether (30 ml) was added dropwise into a solution of lithium aluminum hydride (180 mg) in ether (30 ml) and refluxed for 1 h. The mixture was decomposed with water, acidified with 1M-H_2SO_4 and extracted with ether. After drying the extract was evaporated and separated chromatographically on a silica gel column with a dichloromethane–ether mixture (20 : 1). From the main fractions a substance was obtained with m.p. 132—133°C (from benzene), $[\alpha]_D^{20}$ —30·8° (*c* = 0·32, $CHCl_3$). For $C_{22}H_{28}O_7$ (404·4) calculated: 65·3% C, 7·0% H; found: 65·3% C, 6·7% H. IR spectrum ($CHCl_3$): 3 400 and 3 625 cm^{-1} (hydroxyl), 2 780 cm^{-1} (methylenedioxy), 1 332 and 2 845 cm^{-1} (methoxyl), 1 494, 1 507 and 1 594 cm^{-1} (aromatic ring). CD spectrum (methanol): $\Delta\varepsilon_{277}$ —0·25, $\Delta\varepsilon_{257}$ —0·09, $\Delta\varepsilon_{245}$ —1·17. ^{1}H NMR spectrum is in Tables I and II. Mass spectrum: 77 (24), 135 ($C_8H_7O_2$, 18·6), 151 (17·6), 167 (9·3), 181 ($C_{10}H_{13}O_3$, 82), 182 ($C_{10}H_{14}O_3$, 100), 207 (2·7), 208 (1·7), 219 (3·4), 225 ($C_{12}H_{17}O_4$, 2), 233 (1·7), 237 (2·6), 238 (2·3), 250 ($C_{14}H_{18}O_4$, 4·2), 373 (0·7), 374 (0·6), 404 (M^+, $C_{22}H_{28}O_7$, 37).

Diol *IIb*

Yatein (*Ia*, 60 mg) in ether (10 ml) was added dropwise to a suspension of lithium aluminum deuteride (300 mg) in ether (10 ml) and refluxed for 1 h. The mixture was allowed to stand at room temperature overnight, then decomposed with ^2H_2O, acidified with 50% H_2SO_4 and extracted with ether. After drying the extract was evaporated, dissolved in methanol, reevaporated and chromatographed on silica gel with a mixture of dichloromethane and ether (20 : 1). The diol obtained had m.p. 134·5—135·5°C and it was used for mass spectrometry.

Tribromoyatein *III*

Yatein (*Ia*, 700 mg) was dissolved in chloroform (25 ml). A 0·1M-$KBrO_3$ solution (25 ml), KBr (1 g) and 2M-H_2SO_4 (10 ml) were then added and the mixture shaken for 15 min in the dark. A 10% solution of KI was then added, the mixture shaken and set aside in the dark for about 5 min. It was then titrated with 0·05M-$Na_2S_2O_3$. The aqueous layer was extracted with chloroform and this extract was added to the former chloroform layer. The combined chloroform solutions were washed with water, dried and evaporated. The residue was chromatographed on a column of silica gel with benzene–ethyl acetate (100 : 1) and crystallized from benzene + + ethyl acetate. The product had m.p. 183—184°C and $[\alpha]_D^{20}$ —3·9° (*c* = 0·3, $CHCl_3$). For $C_{22}H_{21}Br_3$ (637·1) calculated: 41·4% C, 3·3% H and 37·7% Br; found: 41·7% C, 3·3% H and 37·8% Br. ^{1}H NMR spectrum is given in Tables I and II. Mass spectrum: 135 (29·1), 165 (58·5), 166 (19·1), 213 (83·3), 215 (79·1), 238 (7·4), 343 (8·5), 245 (5·7), 259 (11·7), 261 (12·1), 275 (7·1), 277 (7·1), 294 (3·1), 296 (6), 298 (4·6), 315 (3·5), 316 (8·2), 317 (5·3), 318 (11), 319 (4·3), 337 (19·1), 339 (39·7), 341 (21·6), 397 (2·8), 475 (3·2), 476 (6·9), 477 (5·7), 478 (7·4), 554 (51·4), 556 (100), 558 (39·2), 634 (8·5), 636 (24·3), 638 (24·5), 640 (9·2).

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