THE BENZOFURANS OF *ISOCOMA WRIGHTII*.[1-3] STRUCTURE AND STEREOCHEMISTRY.

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ABSTRACT.—Fractionation and chromatography of the anti-tumor chloroform portion of the ethanol extract of *Isocoma wrightii* led to the isolation of the family of benzofurans: toxol (2S, 3R-2-isopropenyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran) (**4**); toxyl angelate (**6**); tremetone (2S-isopropenyl-5-acetyl-2,3-dihydrobenzofuran) (**1**); dehydrotremetone (**3**); 2,5-diacetylbenzofuran (**5**) and toxethol [2S-isopropenyl-3R-hydroxy-5-1(1'-ethoxyethyl)-2,3-dihydrobenzofuran] (**7**). In addition, a number of steroids, triterpenes, sesquiterpenes, monoterpenes, and hydrocarbons were isolated. The relative and absolute configurations of the natural 2,3-dihydrobenzofurans have been determined by chemical correlations, nmr analysis of the C-2, C-3 proton coupling constants, and an X-ray analysis of racemic *cis*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran. The isomeric racemic *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran was converted into racemic dihydrotoxol. Racemic *cis* and *trans* 2-methyl, 2-ethyl, and 2-n-propyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran were synthesized and their ^{1}H nmr spectra, particularly the C-2, C-3 coupling constants, are discussed. Toxethol was synthesized from toxol, and saponification of toxyl angelate gave toxol. Ozonolysis of *cis* and *trans*-2-isoproyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran gave, respectively, *threo* and *erythro*-2,3-dihydroxy-4-methylpentanoic acid, which were stereo-specifically synthesized.

"Milk sickness," a disease dreaded in the nineteenth century, was shown to be due to the animal's ingestion of *Eupatorium rugosum* and *Isocoma wrightii* (1, 2). The toxic principle was reported to be tremetol, an unsaturated alcohol (2–5). It was later found (6) that tremetol was not a pure substance but a mixture composed of a sterol fraction and a ketone fraction (7). Only the ketone fraction gave Couch's (5) characteristic sulfuric acid color test for "tremetol." The ketone fraction was separated into three new benzofurans: tremetone (**1**), hydroxytremetone (**2**), and dehydrotremetone (**3**). At about the same time, we reinvestigated "rayless goldenrod tremetol" (8) and isolated from it a ketone fraction containing dehydrotremetone (**3**) and a new benzofuran, toxol (**4**). The isolation of "tremetol" involves an intensive alkaline saponification, and we have now found that the non-saponified hexane and alcoholic extracts of rayless goldenrod contain the related benzofurans 2,5-diacetylbenzofuran (**5**), toxyl angelate (**6**), and toxethol (**7**).[6] Careful chromatographic techniques have revealed that rayless goldenrod also contains tremetone (**1**).

[1]This plant was formerly known as *Haplopappus* (*Aplopappus*) *heterophyllus*. See D. S. Correll and M. C. Johnston, "Manual of Vascular Plants of Texas," Texas Research Foundation, Renner, Texas, 1970.

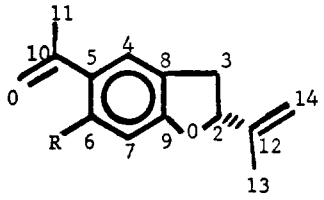
[2]Preliminary accounts of some of this work were presented as follows: L. H. Zalkow, J. R. Novak, Jr., B. Ekpo and R. N. Harris, III, 28th Southeast Regional Meeting, American Chemical Society, Gatlinburg, Tenn., Oct. 27–29, 1976. L. H. Zalkow, J. R. Novak, Jr., B. Ekpo and R. N. Harris, III, 18th Annual Meeting, American Society of Pharmacognosy, Seattle, Washington, August 11–13, 1977.

[3]A portion of this work appeared in a preliminary communication (39).

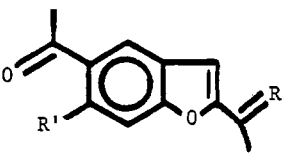
[4]To whom inquiries should be made.

[5]Taken in part from: J. R. Novak, Jr., Ph.D. Dissertation, Georgia Institute of Technology, Atlanta, Georgia, 1977; E. Keinan, Master's Thesis, Ben-Gurion University of the Negev, Beer-Sheva, Israel, 1972; L. T. Ranming, Master's Thesis, Oklahoma State University, Stillwater, Oklahoma, 1963.

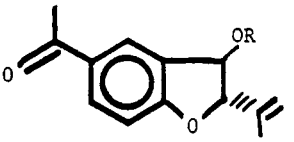
[6]We have coined the trivial name toxethol for **7** to maintain consistency with the trivial name toxol for **4**.



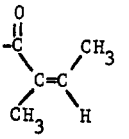
(**1**) R=H
(**2**) R=OH



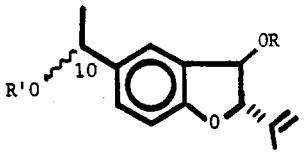
(**3**) R=CH_2, R'=H
(**5**) R=O, R'=H
(**8**) R=CH_2, R'=OH



(**4**) R=H
(**6**) R=



(**9**) R=$COCH_3$



(**7**) R=H, R'=CH_2CH_3
(**10**) R=$COCH_3$, R'=H
(**11**) R=$COCH_3$, R'=CH_2CH_3

While none of the above-mentioned benzofurans have been implicated as the causative agents of "trembles" in higher animals, some of them show biological activity. Toxol (**4**) and dehydrotremetone (**3**) are bacteriostitic against a number of bacteria (8); and tremetone (**1**), dehydrotremetone (3), and hydroxytremetone (**2**) are toxic to goldfish (7). A recent investigation showed that "white snakeroot tremetol" causes ketoacidosis in chicks (9). The chloroform fraction of the chloroform-water partition of the ethanol extract of rayless goldenrod shows antitumor activity against P388 lymphocytic leukemia tumors.[7] Toxol (**4**) and toxyl angelate (**6**) show weak antitumor activity in this system.[7]

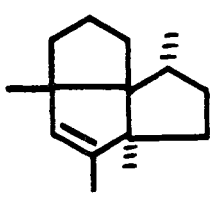
Until recently, benzofurans comprised a very small group of natural products. The first member of this group to be reported was euparin (**8**), (6-hydroxydehydrotremetone) from *Eupatorium purpurem* (10). A recent compendium of natural products (11) classified these simple benzofurans into two separate groups: the C_6C_2 compounds (shikimate derived) i.e. tremetone (**1**), dehydrotremetone (**3**), and toxol (**4**) and the phloroglucinols (polyketide derived) i.e. euparin (**8**) and hydroxytremetone (**2**). This division appears to be unjustified since the only biosynthetic study of this family thus far reported has shown that the acetophenone moiety of dehydrotremetone was derived from acetate via the polyacetate pathway (12). As expected, the furan ring and its side chain were formed from an isoprenoid compound. Recently, benzofurans of this type were found to occur more commonly than previously suspected in the Compositae family, and approximately forty such compounds are now known (13–31).

Synthesis of the benzofurans such as dehydrotremetone (**3**) which contain a double bond at C–2, C–3 has not posed much difficulty (32–34). Synthesis of 2,3-dihydrobenzofurans such as tremetone (**1**) also seems to be readily accom-

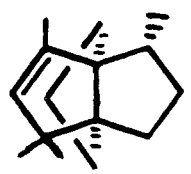
[7]Screening by NCI of the National Institutes of Health.

plished (35, 36). On the other hand, synthesis of 2,3-dihydrobenzofurans containing an oxygen substituent at C–3, such as toxol (**4**), has not appeared in the literature. However, the closely related *cis* and *trans* dihydrotoxols have been synthesized (37). Relative stereochemical assignments in the 2-oxygenated benzofurans of the toxol (**4**) group have been largely based on examination of the C–2, C–3 proton coupling constants in their nmr spectra (13–31). The absolute configuration of tremetone (**1**) and toxol (**4**) at C–2 is based on chemical correlations with rotenone and methyl D(+) malate (38), while the absolute configuration of toxol (**4**) at C–3 is based on an X-ray analysis and a chemical degradation of a synthetic intermediate (39). Further details of these stereochemical assignments are discussed later in this paper. No other independent absolute configurational studies of this family of benzofurans have been reported.

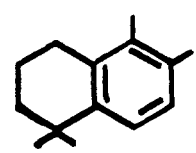
Rayless goldenrod (*I. wrightii*) has been an unusually rich source of secondary plant metabolites of diverse structures. Thus, in addition to the above mentioned benzofurans, the novel steroids 5α-androstane-3β,16α,17α-triol (40) and stigmasta-8(14),22-dien-3β-ol (41) were isolated, and we have now identified stigmasta-5,22-dien-3β-ol and stigmasta-8(14)-en-3β-ol. From the hexane extract, we have identified the triterpenes friedelin, friedelan-3α-ol(42), friedelan-3β-ol, and squalene and the diterpene phytol. In the sesquiterpene family we have found the relatively common β-caryophyllene and β-caryophyllene oxide and the highly unusual isocomene (**12**) (43), modhephene (**13**) (44), and the tetrahydronaphthalene **14** (45).



(**12**)



(**13**)



(**14**)

Isocomene and modhephene represent new sesquiterpenoid skeleta, while **14** is a nordrimane sesquiterpene (C_{14}). Among the monoterpenes, we have identified (+) limonene, (−) carvone, (−) borneol, and bornyl acetate (45). The hexane extract of rayless goldenrod yielded a complex hydrocarbon fraction. After chromatography nonacosane, henitriacontane, and tritriacontane were isolated, and several others were tentatively identified by glc. Among the fatty acids, stearic acid was isolated. Hexanoic, octanoic, lauric, myristic, palmitic, and linoleic acid were identified by glc of their methyl esters. Finally, a family of fatty alcohols has been isolated but not yet completely characterized.

DISCUSSION

The benzofurans dehydrotremetone (**3**), toxyl angelate (**6**), 2,5-diacetylbenzofuran (**5**), toxethol (**7**), and toxol (**4**) were isolated, in the order given, by chromatography of the acid-free aqueous methanol fraction on acid-washed alumina (activity grade III). This fraction was obtained as follows: The dried leaves and flowers of *I. wrightii* were defatted with hexane, then extracted with 95% ethanol. The latter extract, after removal of solvent, was partititoned between chloroform and water. The chloroform fraction, after removal of solvent, was further partitioned between aqueous methanol (1:9) and hexane. The aqueous methanol fraction, after removal of the solvent, was dissolved in ether. This

solution was washed with cold 5% sodium hydroxide solution. Glc indicated, in addition to the above mentioned benzofurans (**3–7**), the presence of tremetone (**1**). Tremetone was isolated by the following procedure. The ethanol extract of the above-ground plant material, after initial defatting with hexane, was saponified with potassium hydroxide (~10%) in aqueous methanol (1:1). The ether-soluble portion of this mixture was a "red jelly" analogous to the original rayless goldenrod "tremetol" of Couch (5).

This "red jelly" was separated into ketone and non-ketone fractions using Girard's T reagent. Glc of the ketone fraction showed three major components of increasing retention time in the ratios of 1.3:1:5.3. These components were isolated by chromatography on silica gel and identified as dehydrotremetone (**3**), tremetone (**1**) and toxol (**4**), respectively. Toxyl angelate (**6**) was most readily isolated from the original hexane extract of the above-ground plant material as follows. The hexane extract was steam distilled and the ether-soluble nonvolatile residue was distributed between benzene and aqueous ethanol. The benzene fraction was washed with a cold 5% sodium hydroxide solution and chromatographed on alumina (activity grade II) and then silica gel to give toxyl angelate (**6**).

The structure of toxyl angelate (**6**) was surmised from analysis of ^1H and ^{13}C nmr spectra and its mass spectrum (see tables 1, 2 and 3) and confirmed by its

TABLE 1. *^1H-Nmr data of the isolated benzofurans.*[a]

C	(3)	(5)	(1)	(4)	(6)	(7)
2	—	—	5.27 (t, 9.0)	5.05 (d, 3.7)	5.00 (d, 2.5)	5.09 (m)
3	6.67 (s)	7.57 (s)	a) 3.08 (m) b) 3.36 (m)	a) 4.97 (b) b) 3.87 (b) (OH)	6.17 (d, 2.5)	4.88 (m)
4	8.17 (d, 2.0)	8.35 (d, 1.7)	7.84 (m)	8.02 (d, 2.0)	8.04 (d, 2.0)	7.32 (m)
5	—	—	—	—	—	—
6	7.93 (dd, 2.0, 8.7)	8.13 (dd, 1.7, 9.0)	7.84 (m)	7.88 (dd, 2.0, 8.5)	7.93 (dd, 2.0, 8.5)	7.24 (dd, 2.0, 8.5)
7	7.43 (d, 8.7)	7.62 (d, 9.0)	6.81 (d, 9.0)	6.88 (d, 8.5)	6.89 (d, 8.5)	6.84 (d, 8.5)
8	—	—	—	—	—	—
9	—	—	—	—	—	—
10	—	—	—	—	—	4.40 (q, 6.5)
11	2.22 (s)	2.68 (s)	2.53 (s)	2.51 (s)	2.47 (s)	1.40 (d, 6.5)
12	—	—	—	—	—	—
13	1.80 (s)	2.63 (s)	1.76 (bs)	1.73 (s)	1.71 (s)	1.76 (s)
14	a) 5.23 (bs) b) 5.83 (bs)	—	a) 5.09 (bs) b) 5.18 (bs)	a) 4.93 (bs) b) 5.08 (bs)	a) 4.90 (d, 1.0) b) 5.03 (d, 1.0) R=angeloyl 1.92 (dq, 7.3, 1.5) 6.06 (qq, 7.3, 1.5) 1.77 (dq, 1.5, 1.5)	a) 5.09 (m) b) 4.88 (m) 15) 3.32 (q, 7.0) 16) 1.17 (t, 7.0)

[a]Spectra were run at 99.95 MHz in $CDCl_3$ solution using a JEOL PFT-100 instrument in FT mode; chemical shift values are expressed in δ values (PPM) relative to TMS.

hydrolysis to the known toxol (**4**). Likewise, the structure of toxethol (**7**), partially suggested from its spectral properties, was firmly established, except for the configuration at C–10, by its synthesis from toxol (**4**) as follows. Acetylation gave toxyl acetate (**9**), which on careful reduction with $NaBH_4$ gave the hydroxyacetate **10**. In turn, **10** was ethylated with ethyl iodide and silver oxide to give **11**. Finally, saponification of the latter gave synthetic toxethol (**7**) identical by nmr and ir spectra with the natural material and having a similar plain negative ord curve. The structure of 2,5-diacetylbenzofuran (**5**) was readily arrived at when it was first isolated in 1963 by comparison of its ^1H nmr and ir spectra with those

TABLE 2. *^{13}C-Nmr data of the isolated benzofurans.*[a]

C	(3)	(5)	(4)	(6)	(1)	(7)
2	156.4 (s)[c]	152.8 (s)	94.3 (d)	90.9 (d)	86.5	93.4 (d)
3	110.2 (d)	113.1 (d)	75.3 (d)	76.3 (d)	33.9	76.2[b]
4	124.5 (d)[b]	127.5 (d)[b]	131.7 (d)[b]	132.5 (d)[b]	130.3[b]	122.6 (d)[d]
5	131.6 (s)	126.4 (s)	128.5 (s)	126.6 (s)	126.9	127.5 (s)[c]
6	121.4 (d)[b]	124.3 (d)[b]	126.3 (d)[b]	127.6 (d)[b]	124.8[b]	128.0 (d)[d]
7	102.5 (d)	111.8 (d)	109.4 (d)	109.5 (d)	108.3	109.3 (d)
8	128.4 (s)	132.8 (s)	130.1 (s)	130.8 (s)	129.9	136.2 (s)[c]
9	157.5 (s)[c]	156.7 (s)	163.5 (s)	163.9 (s)	163.3	158.6 (s)
10	196.3 (s)	195.4 (s)	196.3 (s)	194.8 (s)	195.3	77.1[b]
11	26.4 (q)	26.5 (q)[c]	26.2 (q)	26.1 (q)	26.1	23.9 (q)
12	131.9 (s)	186.7 (s)	140.5 (s)	139.6 (s)	142.6	141.1 (s)
13	19.0 (q)	26.3 (q)[c]	17.4 (q)	17.6 (q)	17.0	15.2[c]
14	113.6 (t)	———	112.2 (t)	113.1 (t)	111.9	111.6 (t)
					R = Angeloyl	15) 63.3 (t)
					166.3 (s)	16) 17.4[c]
					124.7 (s)	
					20.3 (q)	
					138.8 (d)	
					15.8 (q)	

[a]Spectra were run at 25 MHz in $CDCl_3$ solution using a JEOL PFT-100 instrument in FT mode; chemical shift values are expressed in δ values (PPM) relative to TMS. b, c, d, e, assignments may be reversed.

of samples prepared by oxidation of dehydrotremetone (**3**), toxol (**4**), and toxol acetate (**9**). It was also synthesized from synthetic tremetone (**1**) prepared according to Bonner (36) by hydroxylation of the double bond and oxidative cleavage to give the diketone (38) and, finally, dehydrogenation with 5% Pd on carbon. We were surprised to find that samples of isolated dehydrotremetone (**3**) standing under normal conditions in vials in the laboratory were oxidized to 2,5-diacetylbenzofuran (**5**). This surprising result may arise from dehydrotremetone's acting as its own photosensitizer leading to a light-catalyzed (2+2) cycloaddition of singlet oxygen to the isopropenyl double bond of dehydrotremetone and, finally, cleavage of this four-membered intermediate to give 2,5-diacetylbenzofuran and formaldehyde.

TABLE 3. *Major peaks from the mass spectra of the isolated benzofurans.*

Compound	M^+ *m/e*	Misc.	$[M^+-CH_3]$	$[M^+-(CH_3-C\equiv O)]$	$[CH_3-C\equiv O^+]$
(**3**)	200 (52%)		*m/e* 185 (100%)	*m/e* 157 (40%)	*m/e* 43 (31%)
(**5**)	202 (41%)		*m/e* 187 (100%)	*m/e* 159 (17%)	*m/e* 43 (14%)
(**1**)	202 (91%)		*m/e* 187 (53%)	*m/e* 159 (50%)	*m/e* 43 (100%)
(**4**)	218 (28%)	*m/e* % 187 55 185 43 163 34 162 50	*m/e* 203 (32%)	*m/e* 175 (16%)	*m/e* 43 (100%)
(**6**)	300 (3%)	*m/e* 200 (**3**)[⊖] 100% *m/e* 83 angeloyl 90%	*m/e* 200-CH_3 *m/e* 185 (80%)	*m/e* 200-CH_3-C≡O *m/e* 157 (19%)	*m/e* 43 (58%)
(**7**)	248 (20%)	*m/e* 215 (24%)	*m/e* (200) (100%)		

In table 1 we have tabulated the ^1H nmr spectral data on the benzofurans isolated from *I. wrightii,* and in table 2 we have tabulated the ^{13}C nmr data on these compounds. These ^1H data of these benzofurans are consistent with those reported by Bohlmann *et al.* (13–21) for related structures and, together, provide a rather complete set of data for these naturally-occurring benzofurans. The ^{13}C nmr data in table 2 is the first such report. In table 3 we have tabulated the major fragments observed in the mass spectra of the isolated benzofurans. In every case, except that of toxol, the parent ion or M^+-CH_3 is the base peak or, at least, a very large peak. The ms of toxol is thus unusual, and we are uncertain of the cause of the peaks at *m/e* 187 and 185. The base peak (*m/e* 200) in toxyl angelate (**6**) corresponds to the loss of angelic acid, as expected.

Toxol (**4**) was originally assigned to 2S,3S configuration and, as mentioned previously, the configurational assignment at C–2 was correlated with several compounds of known absolute configuration. The assignment at C–3 was based on a single experimental observation, namely, the ozonolysis of toxol to yield, supposedly, (+) tartaric acid (38). A number of years ago we reported the synthesis of racemic *trans* and *cis*-2-isopropyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran (37). The isomer which was spectrally identical with dihydrotoxol was assigned a *cis* relationship at C–2, C–3 based on the above-mentioned ozonolysis of toxol to (+) tartaric acid. This assignment led to an unexpected consequence. In synthetic dihydrotoxol and all of its precursors, the coupling constant for the vicinal C–2, C–3 protons was consistently smaller (J = 3–4.5 vs 5–6 Hz) than in the isomeric *trans* series, in apparent violation of the Karplus equation. This anomaly led us to reinvestigate the configuration of toxol at C–3. In a preliminary report (39), we presented the conclusion that the absolute configuration of toxol was in fact *2S, 3R* and, therefore, *trans* rather than *cis* as previously assumed, and there was, therefore, no violation of the Karplus equation. This preliminary report was based on the X-ray analysis of the synthetic 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrotoxol (mp 112–113°) (**15**) belonging to the series not related to toxol. At that time (1972), the X-ray data was not well refined. We have now repeated the X-ray analysis using more sophisticated means as follows.

Epoxy cement was used to mount a crystal of (**15**) with the approximate dimensions .45 x .45 x .25 mm on a glass fiber so that the longest crystal dimension was approximately parallel to the fiber axis. The crystal was coated with epoxy cement since an unprotected crystal decomposed in the atmosphere. Unit cell parameters and the orientation matrix were determined on a Syntex $P2_1$ four circle diffractometer equipped with a graphite monochromator using MoKα radiation. Unit cell parameters obtained were a = 12.808(8)Å, α = 104.35(5)°, and V = 1862(a)Å. The calculated density of 1.38 g cm^{-1} for six formula units per unit cell agrees with the experimental density of 1.36 g cm^{-1} measured by the flotation method using aqueous zinc chloride. The crystal belonged to the rhombohedral system, and space group $R\bar{3}$ (No. 148) (46) was assumed. Successful refinement of the structure confirmed this choice. Intensity data were collected using θ–2θ scans with X-ray source and monochromator settings identical to those used for the determination of the unit cell parameters. From a total of 2121 unique reflections collected out to 2θ = 50°, 793 were accepted as statistically above background on the basis that F was greater than 5 σ(F). Computations were performed using standard programs (47). For structure factor calculations the scattering factors were taken from Cromer and Mann's tabulation (48). The agreement factors were defined in the usual way as

$$R = (\Sigma||F_o|-|F_c||)/(\Sigma|F_o|)$$

and

$$R_w = [\Sigma(|F_o|-|F_c|)(w^{1/2})/\Sigma(|F_o|)(w^{1/2})]$$

In all least-squares refinements, the quantity minimized was $w(|F_o|-|F_c|)^2$. A weighting scheme based on counting statistics $w = 1.00/(\sigma(F)^2+.01\ F^2)$ was employed for calculating R_w in the least-squares refinement. The structure was solved using the automatic centrosymmetric direct methods section of SHELX-76. The E-map generated by this program contained all non-hydrogen atoms except the isopropyl group. The carbons in the isopropyl group were located from a subsequent difference Fourier synthesis. Hydrogen positions were calculated using the capabilities of the SHELX-76 program. Carbon-hydrogen bond lengths were fixed at 1.08Å, the hydrogen temperature factors were varied, and the hydrogen positions were recalculated before each cycle of refinement. The final parameters varied included an overall scale factor, positional parameters for the oxygens and carbons, anisotropic thermal parameters for the oxygens, and bromine and isotropic thermal parameters for the carbons and hydrogens (80 variables; 793 observations). The final R factor was .102 and $R_w = .113$, full-matrix least-squares refinement.

Table 4 shows the coordinates of the oxygen, carbon, and hydrogen atoms. Figure 1 shows a computer-generated picture of the molecule, clearly indicating that this material (m.p. 112–113°) is *cis*-2-isopropyl-3-hydroxy-5-bromo-2,3-di-

TABLE 4. *Coordinates of the atoms (standard deviations are indicated in parenthesis and refer to the last decimal place).*

Atom	X	Y	Z	U11	U22	U33	U23	U13	U12
BR	.8897(2)	.3537(3)	.9364(2)	.108(2)	.194(3)	.075(2)	.026(2)	.015(1)	.063(2)
01	.4298(8)	.1167(8)	.5878(8)	.069(7)	.064(6)	.072(7)	.032(5)	.039(6)	.025(5)
02	.5565(8)	.3269(8)	.5136(8)	.087(7)	.068(6)	.078(7)	.049(6)	.055(6)	.046(6)
Atom	X	Y	Z	U					
C2	.453(1)	.126(1)	.483(1)	.063(4)					
HC2	.465(1)	.053(1)	.431(1)	.09(5)					
C3	.563(1)	.219(1)	.518(1)	.047(3)					
HC3	.611(1)	.198(1)	.462(1)	.04(3)					
C4	.615(1)	.227(1)	.642(1)	.047(3)					
C5	.719(1)	.282(1)	.715(1)	.059(4)					
HC5	.784(1)	.330(1)	.689(1)	.05(3)					
C6	.744(1)	.278(1)	.828(1)	.069(4)					
C7	.650(2)	.214(2)	.855(2)	.085(6)					
HC7	.667(2)	.209(2)	.941(2)	.5(2)					
C8	.544(1)	.162(1)	.780(1)	.070(4)					
HC8	.474(1)	.117(1)	.802(1)	.06(4)					
C9	.532(1)	.168(1)	.673(1)	.055(3)					
C10	.350(1)	.131(2)	.401(1)	.079(5)					
HC10	.334(1)	.208(2)	.439(1)	.08(4)					
C11	.374(2)	.128(2)	.289(1)	.087(6)					
HC11	.301(2)	.131(2)	.228(1)	.16(5)					
HC11	.392(2)	.051(2)	.256(1)	.16(5)					
HC11	.447(2)	.201(2)	.306(1)	.16(5)					
C12	.241(2)	.035(2)	.376(2)	.103(6)					
HC12	.224(2)	.037(2)	.455(2)	.23(7)					
HC12	.251(2)	−.046(2)	.339(2)	.23(7)					
HC12	.170(2)	.045(2)	.318(2)	.23(7)					

The form of the thermal ellipsoid expression is $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}-2U_{12}hka^*b^*+2U_{13}hla^*c^*+2U_{23}klb^*c^*)]$.

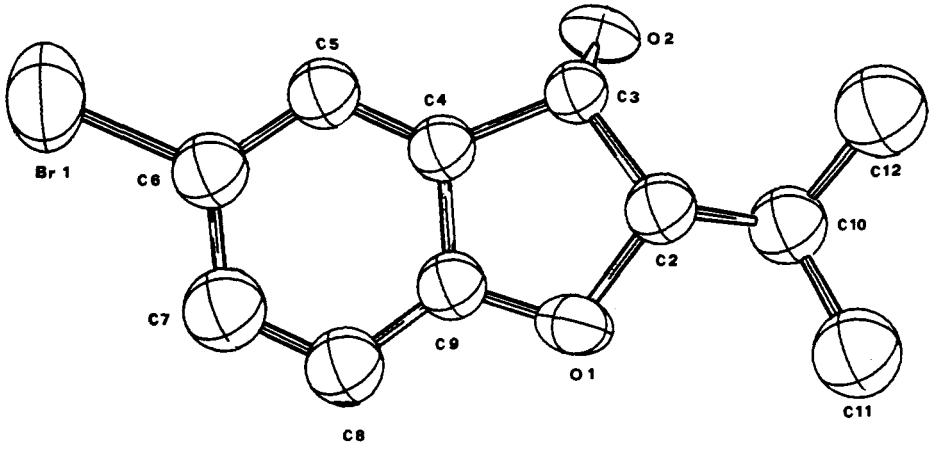
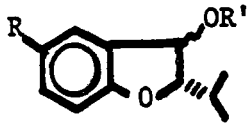
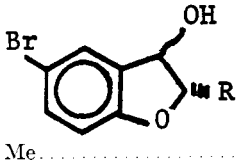


FIG. 1

hydrobenzofuran. Therefore, the isomeric substance (mp 44.5–45°) which was converted into dihydrotoxol must be *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran. Dihydrotoxol, toxol, toxol angelate and toxethol must be *trans* at C–2, C–3 and have the absolute configurations indicated in **4**, **6** and **7** since the absolute configuration at C–2 in toxol is well established (38).

In table 5 we have tabulated the C–2, C–3 vicinal proton nmr coupling constants of the various synthetic intermediate *cis* and *trans* dihydrobenzofurans prepared in our laboratory in the synthesis of dihydrotoxol (37), the natural pro-

TABLE 5. *C-2, C-3 Vicinal ^1H coupling constants.*

Compound	$J_{2,3}$ Hz		Ref.
	Cis	Trans	
			
R=Br, R'=H	(**15**) 5.5	(**16**) 4.2	(37)
R=Br, R=$COCH_3$	(**17**) 6	(**18**) 3.5	(37)
R=CO_2H, R'=H	—	(**19**) 4.5	(37)
R=$COCH_3$, R'=H	(**20**) 6	(**21**) 4	(37)
			
R=Me	(**22**) 6.5	(**23**) 3.5	
R=Et	(**24**) 6.0	(**25**) 3.5	
R=n-C_8H_7	(**26**) 5.5	(**27**) 3.7	
toxol (**4**)	—	3.7	
toxyl acetate (**9**)	—	3	
toxyl angelate (**6**)	—	2.5	
toxethol (**7**)	—	~3	
(**10**)	—	3	
(**11**)	—	3	

ducts toxol (**4**), toxyl angelate (**6**) and toxethol (**7**), and their derivatives (**9**), (**10**) and (**11**). Unfortunately in the case of toxethol (**7**) the chemical shift of one of the olefinic protons overlapped that of the C–2 proton, while the other olefinic proton overlapped that of the C–3 proton in a way that we could not unequivocally determine J_{213} from our spectra. However, we could estimate a maximum value of $J_{213}\sim 3$ Hz, and the corresponding acetate (**11**) and derivative (**10**) clearly showed values of J_{213} of 3 Hz. We also prepared a series of 2-alkyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans (**22**–**27**). As can be seen from table 5, all of the *cis* and *trans* isomers are clearly distinguishable in each case by their differences in J_{213}. On this basis the natural products toxol (**4**), toxyl angelate (**6**) and toxethol (**7**) were determined to be *trans*.

Chemical support was obtained for the stereochemical assignments of the synthetic isomeric racemic 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans **15** and **16** as follows (see figure 2). Ozonolysis of the isomer of mp 112–113° (**15**) yielded *threo*-2,3-dihydroxy-4-methylpentanoic acid (**28**), whereas ozonolysis of the isomer of mp 44.5–45° (**16**) gave the corresponding *erythro* acid (**29**). The nmr spectra of the crude ozonolysis products from isomeric **15** and **16** clearly showed that in the former case only *threo* **28** was produced while in the latter case

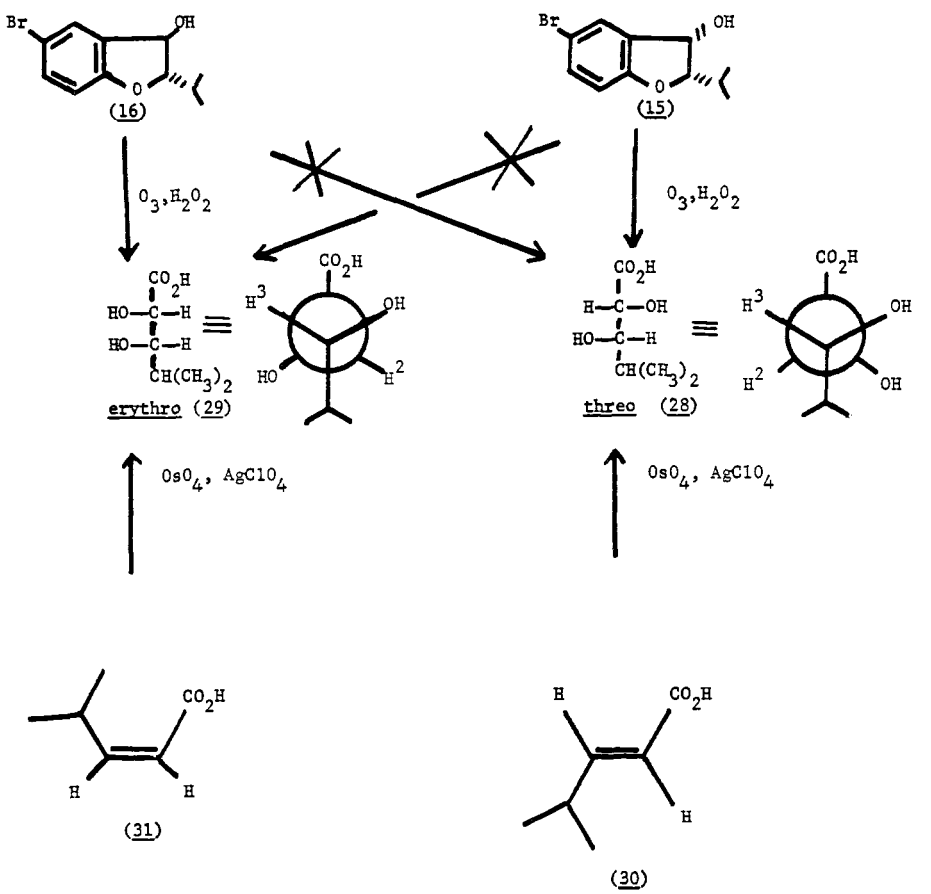


FIG. 2. *Relative configurations of the 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans.*

only *erythro* **29** was formed by adding the isomer not produced in the ozonolysis to the nmr tube to show that it could be detected in small amounts. Glc analysis also showed that each ozonolysis produced a single acid product. The authentic samples of racemic *threo* (**28**) and *erythro*-2,3-dihydroxy-4-methylpentanoic (**29**) acids were prepared by stereospecific *cis* hydroxylation of trans-4-methyl-2-pentenoic acid (**30**) and *cis*-4-methyl-2-pentenoic acid (**31**), respectively, as illustrated in figure 2. While *threo* and *erythro* 2,3-dihydroxy-4-methylpentanoic acids

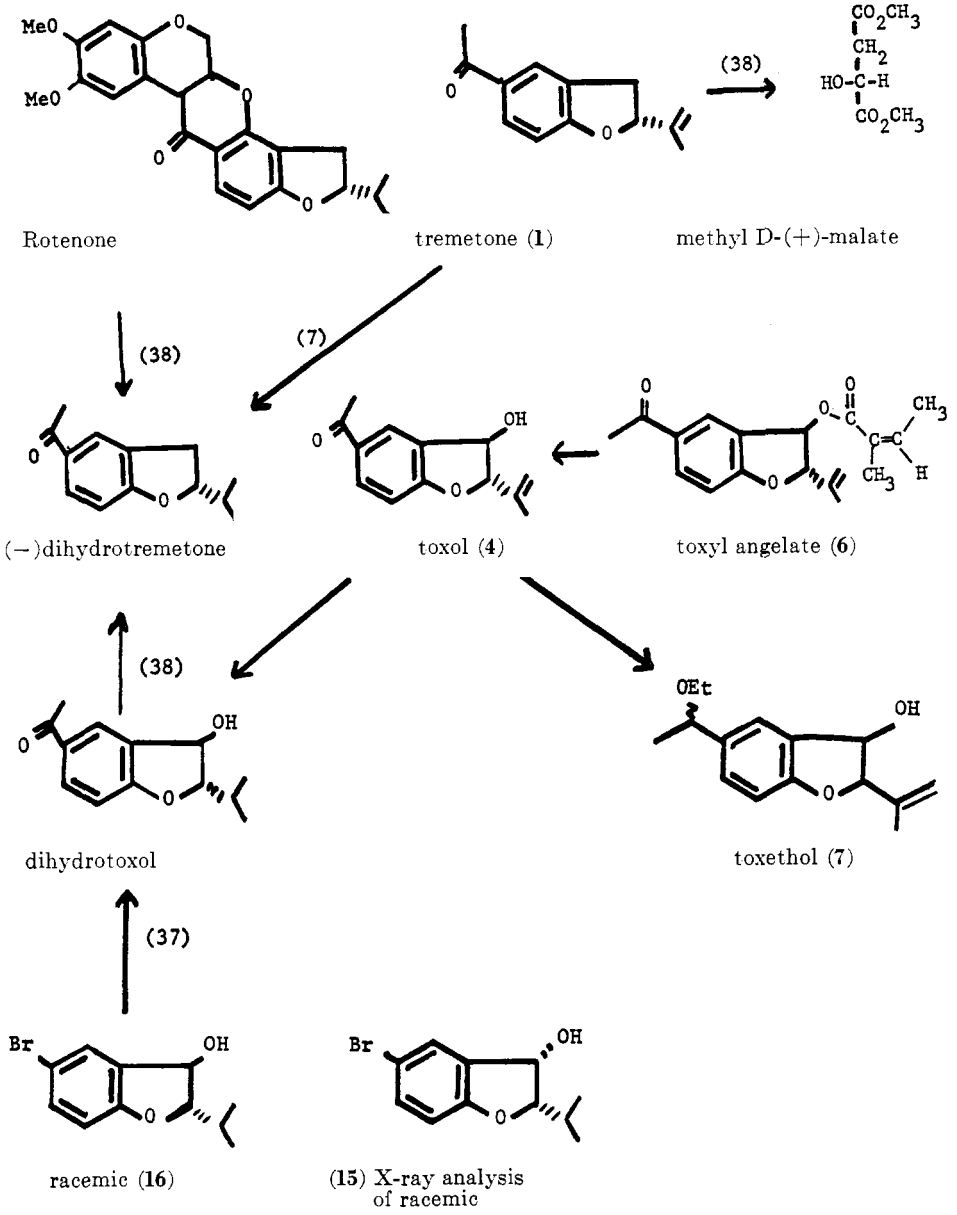


FIG. 3. *The absolute configuration of toxol and its congeners.*

were prepared by stereospecific syntheses, their nmr spectra further confirmed the configurational assignments. Thus, the averaged spectrum (in D_2O) of **28** (preferred conformer indicated in figure 2) showed $J_{2,3}=2$ Hz while that of **29** (preferred conformer indicated in figure 2) showed $J_{2,3}=5.5$ Hz at room temperature as expected for the *threo* and *erythro* isomers, respectively.

The one final inconsistency in this story of the configuration of toxol and related substances at C–3 remains the reported isolation of (+) tartaric acid in the ozonolysis of toxol (38). We recently attempted to repeat this experiment, but, due to the short supply of natural toxol, we began with the more abundant toxyl angelate (2.5 g), which was hydrolyzed to give toxol (1.8 g). The toxol was then ozonized as previously described (38). This time, we could detect no tartaric acid in the ozonolysis product based on nmr and hplc analysis by comparison with an authentic sample. In addition, we could not detect the presence of any of the possible precursor 2-keto-3,4-dihydroxypentanoic acid. Thus, we must consider the original report of the formation of (+) tartaric acid as an unexplained erroneous result. Figure 3 summarizes the absolute configurational relationship of toxol and its congeners.

EXPERIMENTAL[5]

ISOLATION OF DEHYDROTREMETONE (**3**), TOXYL ANGELATE (**6**), 2,5-DIACETYLBENZOFURAN (**5**), TOXETHOL (**7**), AND TOXOL (**4**).—The above-ground parts of rayless goldenrod (*Isocoma wrightii* (Gray) Rydb.) were collected in the vicinity of Artesia, New Mexico, in October 1968. The material was air dried, ground, and stored in sealed bags until extracted in 1974. Twelve kilograms of the plant material were extracted with 19 liters of hexane in Soxhlets for 2 days. Removal of the hexane with a rotary evaporator gave 250 g of extract (fraction A). Extraction of the marc in a similar manner with 95% ethanol gave 275 g of ethanol extract (fraction B). Fraction B was partitioned between chloroform and water (2 liters each). The chloroform layer was washed with a saturated brine solution then dried over anhydrous sodium sulfate. The chloroform was then removed *in vacuo* to give a greenish-brown tar residue (205 g) designated fraction C. Fraction C was partitioned between two liters each of hexane and aqueous methanol (1:9). The hexane layer was concentrated to a green syrup (35 g) and designated fraction D. The aqueous methanol layer was concentrated to 0.5 liter and then one liter of water was added. After the addition of salt to prevent an emulsion, the mixture was extracted with two liters of chloroform. The chloroform extractive was dried over anhydrous sodium sulfate, filtered and then evaporated *in vacuo* to give 163 g of a dark brown tar designated fraction E. Fraction E was dissolved in 1.5 liters of ether, and the resulting solution was extracted with three 300 ml portions of ice cold 5% sodium hydroxide solution and then with a like amount of distilled water. The ether solution was dried over anhydrous sodium sulfate and filtered. After the ether was removed, there remained a viscous greenish-brown syrup (23.5 g) (fraction F). Glc (6', 5% SE-30 column at 175°) indicated fraction F contained dihydrotremetone (**3**), toxyl angelate (**6**), 2,5-diacetylbenzofuran (**5**), toxethol (**7**), toxol (**4**), possibly tremetone (**1**), and a number of unknown constituents.

Chromatography of fraction F (22.4 g) on 1680 g of acid-washed alumina (activity grade III) gave in the hexane-benzene (2:3) eluent, first, 0.57 g dehydrotremetone (**3**) and then 0.28 g toxyl angelate (**6**) (physical and spectral properties are given below). Elution with hexane-ether (1:1) gave 1.1 g of a red-brown solid. The glc of this solid showed a 1:1 ratio of 2,5-diacetylbenzofuran (**5**) and toxethol (**7**). These two substances (**5** and **7**) were separated by further chromatography on 100 g of acid-washed alumina (activity grade II). Elution with chloroform gave 0.32 g of >95% pure 2,5-diacetylbenzofuran (**5**). Further elution with chloroform gave 0.31 g of toxethol (**7**). Elution of the original column of acid-washed alumina (activity grade III) with chloroform-ethyl acetate (9:1) gave 4.65 g of a brown oil. The glc of the oil indicated two components in a ratio of 85:15. On rechromatography of this fraction on 500 g acid-washed alumina (activity grade II), both components were again eluted together with hexane-ethyl acetate (4:1). After the minor, more volatile, component was removed by short path distillation at 110–120°/0.18 mm, almost pure (90–95% by glc) toxol (**4**) remained.

[5]M.p.'s were taken on a Kofler hot stage and are uncorrected. Ir spectra were recorded with a Perkin Elmer 237 B spectrophotometer. ^{1}H nmr spectra were obtained with a Varian A-60D or T60 spectrometer, except where otherwise indicated, with Me_4Si as an internal standard (δ 0); ^{13}C nmr spectra were run on a JOEL-PFT-100 Ft spectrometer. Mass spectra were run on a Hitachi RMV-7 spectrometer; gas chromatography was done with a F&M Biomedical Gas Chromatograph, model 402; and ord spectra were recorded using a Jasco ORD/UV-5 instrument.

DEHYDROTREMETONE (**3**).—Mp 84–85° (from hexane); rpt. mp 87.5–88.5° (7); glc R_t=5.3 min, 6' x ¼" 5% SE-30 glass column at 170°; νKBr 2955, 2925, 1675, 1595, 1565, 1370, 1310, 1275, 1245, 1165, 925, 840, 820 cm^{-1}; λ95% EtOH 254 (4.57), 282 (4.26), 285 (4.22), 294 (4.15), 310 nm (log ε3.67); ^1H nmr-see table 1; ^{13}C nmr-see table 2; mass spec-see table 3.

TOXYL ANGELATE (**6**).—Bp 120–130°/0.1 mm (air bath), sample purified by hplc on a size B EM Reagents silica gel 60 column; R] 0.86, tlc on Eastman chromatogram 13181 silica gel sheet developed with ethyl acetate-hexane (1:1); glc R_t=24 min, 6' x ¼" 5% SE 30 column at 170°; Calc. for $C_{18}H_{20}O_4$: C, 71.98, H, 6.71. Found: C, 72.38, 72.31, 72.25; H, 7.08, 7.07, 7.09; νfilm 1715, 1680, 1645, 1610, 1360, 1295, 1265, 1230, 1150 cm^{-1}; ord (C, 2.31; $CHCl_3$): $[\phi]_{589}=-311.7$, $[\phi]_{550}=-363.6$, $[\phi]_{500}=-467.3$, $[\phi]_{450}=-675.3$, $[\phi]_{400}=-961.0$, $[\phi]_{350}=-1506.5$; $[\alpha]^{25}D-114.7$ (C, 4.62, $CHCl_3$), by polarimeter; ^1H nmr-see table 1; ^{13}C nmr-see table 2; mass spec-see table 3.

Saponification of toxyl angelate with 8% ethanolic potassium hydroxide gave toxol in the neutral fraction, identical by glc, ir, nmr, ord with an authentic sample.

2,5-DIACETYLBENZOFURAN (**5**).—Mp 139–140° (from MeOH), rpt mp 139–10° (8); glc R_t= 6.6 min (same conditions as listed for dehydrotremetone); ν$CHCl_3$ 1680, 1630, 1570, 1365, 1305, 1280, 1160 cm^{-1}; λEtOH 253 (4.61), 286 nm (log ε4.28); ^1H nmr-see table 1. ^{13}C nmr-see table 2. Mass spectrum-see table 3.

2,5-Diacetylbenzofuran was synthesized as follows. Salicylaldehyde was converted into 2-acetylbenzofuran, as previously described (49): yield 45%, mp 70–71°, rpt. mp 72–73° (49). Treatment of 2-acetylbenzofuran with methyl magnesium iodide, as described by Bonner *et al.* (35), yielded 2-benzofuryl-2-propanol, which was then hydrogenated with W-2 Raney nickel catalyst to give 2-(2,3-dihydrobenzofuryl)-2-propanol. The latter was acylated with acetic acid and trifuoroacetic anhydride to give 2-(2,3-dihydro-5-acetylbenzofuryl)-2-propyl acetate, mp 95°, rpt. mp 95–96° (35), in overall yield of 30%. The latter acetate was pyrolyzed at 330° according to Bonner's procedure (36) to give racemic tremetone in quantitative yield. Synthetic racemic tremetone was converted, as previously described (38), into 2,5-diacetyl-2,3-dihydrobenzofuran with 40% yield. A mixture of 0.176 g of 2,5-diacetyl-2,3-dihydrobenzofuran and 0.35 g of 5% Pd on carbon was sealed, under vacuum, in a heavy walled tube which was heated at 215–220° for 1.5 hr. After cooling, the contents of the tube were dissolved in acetone, and the catalyst was removed by filtration. Evaporation gave 0.085 g (50% yield) of a solid which, after recrystallization from methanol, was identical with natural 2,5-diacetylbenzofuran.

TOXETHOL (**7**).—Bp 110–125°/0.1 mm (air bath); R_t=5.5 min, 6' x ¼" 3% OV-17 glass column at 175°; Calc. for $C_{16}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.25; H, 8.52; νfilm 3375, 1615, 1485, 1240, 1100, 1065, 1020, 900, 820, 760 cm^{-1}; λ95% EtOH 291 (3.31), 285 (3.36), 229 nm (log ε 3.78); ord (C, 1.32; $CHCl_3$): $[\phi]_{589}=-47°$, $[\phi]_{550}=-66°$, $[\phi]_{500}=-94°$, $[\phi]_{450}=-132°$, $[\phi]_{400}=-207°$, $[\phi]_{350}=-443°$; ^1H nmr-see table 1; ^{13}C-see table 2; mass spec-see table 3.

Toxethol (**7**) was synthesized from toxol (**4**) as follows. Toxol acetate (**9**) was prepared from toxol (**4**), as previously described, (8) with 96% yield. It had the following physical properties: νfilm 1740, 1680, 1610, 1225–1275 (br); δ($CDCl_3$): 1.77 (3 H,s), 2.11 (3 H,s), 2.56 (3 H,s); mass spec *m/e* 260 (M^+, ~1%), 200 (33%), 185 (44%), 157 (16%), 43 (100%).

To 467 mg of toxol acetate in 5 ml of methanol at 0° was added 34 mg of $NaBH_4$. The reaction mixture was stirred at 0° for 1 hr and then worked up in the usual way. The nmr of the resulting 470 mg of a colorless oil indicated >95% of the desired hydroxyacetate **10**. Bp 125–130°/0.12 mm (air bath); glc R_t=4.3 min, 6' x ¼" 5% SE-30 column at 200°; νfilm 3400, 1740, 1615, 1490, 1235, 1020, 825 cm^{-1}; λ95% EtOH 292 (3.50), 285 (3.58), 226 nm (log ε 3.87); δ($CDCl_3$): 1.47 (d, 3 H, *J*=7 Hz), 1.75 (s, 3H), 2.10 (s, 3 H), 5.01 (m, 3 H), 6.13 (d, 1 H, *J*= 3 Hz); mass spec *m/e* 262 (M^+, 16%), 202 (61%), 187 (51%), 184 (48%), 43 (100%).

A mixture of freshly distilled ethyl iodide (2.5 ml), hydroxyacetate (50 gm), and silver oxide (132 mg) was stirred at 65° for 3 days. The mixture was cooled and chloroform (5 ml) was added. The solution was then filtered, washed successively with aqueous sodium thiosulfate and water, and dried over magnesium sulfate. When evaporated, this solution yielded a pale yellow oil (53 mg). The ^1H nmr of the oil indicated a mixture of desired ether **11** and starting material **10** in a ratio of 3:2. Chromatography on silica gel gave the desired **11** in the hexane-ether (13:2) eluent. It had the following physical properties: νfilm 1740, 1620, 1235, 1115, 1025, 825 cm^{-1}; δ($CDCl_3$) 1.18 (3 H, t, *J*=7 Hz), 1.41 (3 H, d, *J*=7 Hz), 1.79 (3 H, bs), 3.37 (2 H, q, *J*=7 Hz), 4.38 (1 H, q, *J*=7 Hz), 5.03 (4 H, bm), 6.18 (1 H, d, *J*=3 Hz); exact mass Calc. for $C_{17}H_{22}O_4$: 290.152, found: 290.153; *m/e* 290 (M^+, 1%), 230 (50%), 215 (91%), 185 (49%), 43 (100%).

Hydroxy acetate **11** (61 mg) was added to a solution of methanol (2 ml) and 3N sodium hydroxide (0.25 ml). The entire solution was heated at reflux for 1 hr, cooled to room temperature, diluted with brine (10 ml) and then extracted with ether. When washed with brine, dried over magnesium sulfate and evaporated, the solution yielded a pale yellow oil (52 mg); the ^1H nmr of the oil indicated it was ~95% pure toxethol (7). Distillation gave 50 mg of synthetic toxethol, bp 113–120°/0.12 mm (air bath). The ^1H nmr and ir spectra of the synthetic and natural toxethol were essentially indistinguishable and the mass spectra and ord curves of the two were similar but different in relative magnitudes. The latter differences could be

due to instrumental variation or, more probably, to the presence in the synthetic toxethol of the diastereomer, which differed from the natural isomer in configuration at the chiral center bearing the ethoxyl group.

TOXOL (**4**).—Mp 52–53° (from ether-petroleum ether): bp 110°/0.05 mm: glc R_t=8.6 min, 6' x ¼" 5% SE-30 glass column at 170°; $\nu CHCl_3$ 3450, 1670, 1660, 1610 cm^{-1}; ord (C, 2.41, EtOH): $[\phi]_{589}=-50.0°$, $[\phi]_{550}=-63.6°$, $[\phi]_{500}=-90.8°$, $[\phi]_{450}=-140.8°$, $[\phi]_{400}=254.4°$, $[\phi]_{360}=449.7°$; ^1H nmr=see table 1; ^{13}C nmr=see table 2; mass spec-see table 3.

PREPARATION OF THE "RED JELLY" (TREMETOL) AND ISOLATION OF TREMETONE (**1**).—The ethanol plant extract (193 g) fraction B, was dissolved in 1.5 liters of 50% aqueous methanol containing 105 g of potassium hydroxide. After the solution was refluxed for 48 hrs., most of the methanol was removed with the water aspirator. The resulting solution was diluted with 1 liter of water and then continuously extracted with ether for 48 hrs. Drying over anhydrous sodium sulfate and evaporation of the solvent gave 7.4 g of "red jelly." The "red jelly" (4.5 g) was dissolved in 50 ml of methanol containing 4 g of Girard's T Reagent and 1 ml of acetic acid. This solution was refluxed for 1 hr. After being cooled, the solution was poured into 10% sodium carbonate (50 ml) and then extracted with ether. After drying, the ether layer gave 3.6 g of the non-ketone fraction on evaporation. Acidification of the aqueous layer to pH2, extraction with ether, drying, and evaporation gave 0.9 g of the ketone fraction. Glc analysis (6' x ¼" 5% SE-30 glass column at 170°) showed three major components at R_t 5.3, 6.3, and 8.6 min in a ratio of 1.3:1:5.3. Chromatography of the ketone fraction on silica gel gave the compound of R_t=5.3 min in 10% ether in benzene eluent. It was identified as dehydrotremetone (**3**). Further elution with this solvent gave the component of R_t=6.3 min which was identified as tremetone (**1**) (see below for properties). Finally elution with 30% ether in benzene gave the component of R_t=8.6 min, which was identified as toxol.

TREMETONE (**1**).—Mp 38–39° (from ether-benzene): Calc. for $C_{13}H_{14}O_2$: C, 77.20; H, 6.98; Found: C, 77.15; H, 7.01: glc R_t=6.3 min (conditions above); νCCl_4 1672. 1605, 1265, 1230, 905 cm^{-1}; λEtOH 226 (3.94), 279 (3.90), 286 nm (log ϵ 3.90); ord (C, 0.89: EtOH): $[\phi]_{589}=-76°$, $[\phi]_{550}=-92°$, $[\phi]_{500}=-126°$, $[\phi]_{450}=-172°$, $[\phi]_{400}=-287°$, $[\phi]_{350}=-609°$; ^1H nmr-see table 1; ^{13}C nmr-see table 2; mass spec-see table 3.

THE NON-KETONE FRACTION. ISOLATION OF PHYTOL, HENTRIACONTANE, SQUALENE, STIGMASTA-8(14),22-DIEN-3*β*-OL, STIGMASTA-5,22-DIEN-3*β*-OL, STIGMASTA-8(14)-EN-3*β*-OL.—A temperature-programmed glc study of the non-ketone fraction of the "red jelly" indicated that it was a complex mixture separable into low and high boiling components. The non-ketone fraction (34 g) was chromatographed on Merck acid-washed alumina (activity grade I, 380 g). The benzene eluent (4.1 g) was shown by glc to contain predominantly one component. Rechromatography on Merck acid-washed alumina (activity grade I) gave phytol in the benzene eluent with the same spectral properties as previously described (50). Glc R_t=11.5 min (4' x ¼" 3% SE-30 on 100/120 Gas Chrom Q. Program of hold at 150° for 8 min, programmed to rise at 20°/min to 245° and hold at 245°); $\nu CHCl_3$ 3400, 2935, 1650, 1460, 1380, 980 cm^{-1}; $\delta(CDCl_3)$ 0.83 (3 H, d, J=4 Hz), 0.84 (6 H, s), 1.30 (18 H, b), 1.65 (3 H, s), 2.00 (4 H, m), 4.19 (2 H, bd), 5.43 (1 H, m); mass spec *m/e* 296 (M^+, 1%), 71 (100%).

The hexane eluent from the chromatography of the non-ketone fraction gave a 2 g fraction. Glc (column and program as mentioned above) of the fraction indicated the presence of three major components with R_t=15.6, 16.5 and 19 min, respectively. Rechromatography on Merck acid washed alumina (activity grade I) gave the two components of R_t 16.5 and 19 min in the first hexane eluent. Rechromatography on the same type of alumina (ratio of alumina to material 255:1) gave the component of longest R_t in one of the hexane eluents. A final chromatography with a ratio of alumina to substrate of 515:1 yielded this material analytically pure. It was identified as hentriacontane by the following spectral and physical properties (51): glc R_t=5.4 min (6' x ¼", 3% OV 17 glass column at 290°; mp 66–68°, rpt. mp 67° (52); νCCl_4 2950, 2930, 2850, 1460, 1255, 1095 and 1015 cm^{-1}; mass spec *m/e* 436 (M^+, 4%), 57 (100%). Mass spectral and glc studies were used to confirm the structure of hentriacontane ($C_{31}H_{64}$) using authentic samples (Applied Sciences Laboratory & Chem. Sample Co.) of triacontane ($C_{30}H_{62}$) and dotriacontane ($C_{32}H_{66}$). Use of mixed injections showed that hentriacontane fell between the two unknowns in R_t. The mass spectra comparisons clearly showed that the unknown was a straight chain hydrocarbon containing one more CH_2 group than triacontane and one less than dotriacontane.

Further elution with hexane from the column which yielded hentriacontane gave the component with the original R_t=15.6 min. After rechromatography on alumina, this material was obtained pure (glc) and was identified as squalene by comparison of its spectral properties with those reported (53). They were as follows: $\delta(CDCl_3)$: 1.60 (24 H, s, methyls), 1.98 (20 H, bs, methylenes), 5.10 (6 H, bm, olefin protons): mass spec: *m/e* 410 (M^+, 2%), 81 (100%).

THE STEROL FRACTION.—The chloroform eluent of the alumina chromatography of the original non-ketone fraction was found by glc to contain three major high molecular weight compounds (R_t=19.7, 20.4 and 21.4 min, 6' x ¼" 5% SE-30 column at 285°). Rechromatog-

raphy on Merck acid-washed alumina (100:1) gave, in the chloroform eluent, the unseparated three high molecular weight compounds free of low molecular weight contaminants. Further chromatography of the latter on (activity grade III) Merck acid-washed alumina (100:1) gave the component of R_t=20 min in the benzene eluent. It was identified as stigmast-8(14), 22-dien-3β-ol by the following physical and spectral properties (41): glc R_t=20.4 min (column above), 6.3 min (5' x 1/8", 1.5% OV-101 column at 285°); mp 164–165°, rpt mp 165–166 (41); $\nu CHCl_3$ 3600, 2950, 2865, 1460, 1375, 1145, 975 cm^{-1}; $\delta(CDCl_3)$ 0.55 (3 H, s), 0.80 (3 H, s), 3.62 (1 H, bm), 5.09 (2 H, m); mass spec 412 (M^+, 3%), 43 (100%).

The other two high molecular compounds were not separable by chromatography on alumina, silica gel, or silica gel impregnated with silver nitrate. A fraction which showed the three components by glc on the 5% SE–30 column mentioned above showed four components on a 6' x 3/8" 3% OV–17 column at 290° with R_t=14.0, 16.3, 18.4 and 20.1 min. The component with R_t=16.3 min was identified as the above-mentioned stigmasta-8(14),22-dien-3β-ol. Preparative glc on the last-mentioned column gave the components of R_t=14.0 min and 18.4 min, but the component of R_t=20.1 could not be obtained in sufficient quantity for good spectral analysis. The component of R_t=14 min was identified as stigmasta-5,22-dien-3β-ol by comparison with an authentic sample (Aldrich Chemical Co. 5440-9). It had the following physical properties: mp 168–170; mp of authentic sample 170°, mixed mp 168–170°; $\nu CHCl_3$ 3600, 2965, 2875, 1460, 1380, 1030, 974 cm^{-1}; $\delta(CDCl_3)$, 0.70 (3 H, s), 1.01 (3 H, s), 5.09 (2 H, b), 5.35 (1 H, b); *m/e* 412 (M^+, 17%), 55 (100%). The component of R_t=18.4 min was identical by physical and spectral properties to those reported for stigmasta-8(14)-en-3β-ol (54): mp 109–111°, rpt mp 108–111 (54); νCCl_4 3600, 2945, 2855, 1455, 1375, 1035 cm^{-1}; $\delta(CDCl_3)$ 0.54 (3 H, s), 0.80 (3 H, s); *m/e* 414 (100%), 255 (45%).

THE HEXANE EXTRACT. THE BENZOFURANS.—The original hexane extract of rayless golden rod contains a large variety of compounds including benzofurans, sesquiterpenes, triterpenes, sterols, hydrocarbons and long chain alcohols. In a future publication we will discuss this extract in more detail. We present here only the isolation of toxyl angelate and its congeners.

Steam distillation of the hexane extract (400 g) gave an ether soluble non-volatile fraction (360 g) which was partitioned between benzene, ethanol, and water (3:0.75:0.25). The benzene layer was washed with cold 5% sodium hydroxide solution to yield 90 g of a neutral fraction. Chromatography (20 g) on Merck neutral alumina (activity grade II) (50:1) gave, in the hexane-benzene eluent (1:1), 3.8 g of material. Concentration of the solvent precipitated a mixture of friedelin, friedelin-3α-ol, and friedelin-3β-ol. After chromatography on Merck neutral alumina (activity grade II) the mother liquor gave toxyl angelate, which was obtained analytically pure after hplc chromatography on silica gel. The hexane-benzene eluent (1:4) of the original column deposited crystalline 2,5-diacetylbenzofuran, while the chloroform eluent gave toxol.

THE MODIFIED COUCH PROCEDURE (5). ISOLATION OF 2,5-DIACETYLBENZOFURAN.—The ground plant material (6 kg) was extracted with 20 liters of methanol for 72 hr. Removal of the methanol with a water aspirator gave 1 kg of tarry residue, which was taken up in an equal volume of chloroform and then extracted with water. The solvent was removed from the chloroform portion. The resulting residue was dissolved in 50% aqueous ethanol. After filtration, the homogeneous solution was diluted with water until the solution was 30% ethanol. The latter solution was filtered and the filtrate evaporated to give 53 g of residue. This residue was taken up in benzene, and the benzene soluble portion was chromatographed on Merck acid-washed alumina (activity grade I). The ether-benzene (3:2) eluent deposited 4.5 g of 2,5-diacetylbenzofuran, mp 139–140°.

OZONOLYSIS OF *cis* AND *trans*-2-ISOPROPYL-3-HYDROXY-5-BROMO-2,3-DIHYDROBENZOFURAN.—A stream of ozone in oxygen (4.5%) was bubbled, at room temperature, through a solution prepared by dissolving 2 g of *cis*-2-isopropyl-3-hydroxy-5-bromo-2,3-dehydrobenzofuran (mp 105–106°; $J_{2,3}$ 5.5 Hz), prepared as previously described (37), in 30 ml of acetic acid for 24 hr. Hydrogen peroxide (8 ml of 30%) was added and the solution stirred at room temperature for an additional 24 hr; whereupon, palladium on charcoal was added and the solution stirred an additional 2 hr. Filtration and evaporation gave 1.1 g of an oily product. The nmr spectrum of a portion of this crude product showed the presence of only *threo*-2,3-dihydroxy-4-methylpentanoic acid (**29**). There were no peaks present from the isomeric erythro acid **28** (see below for synthesis of **28** and **29**). When some of the synthetic *threo* acid was added to the nmr tube, intensification of the already present peaks resulted. Addition of a small amount of the *erythro* acid resulted in the clear detection of its characteristic peaks in the spectrum. Glc (3% OV–17 column at 136°) of the silanized ($(CH_3)_3$ SiCl) product showed only a peak with the same retention time as the synthetic *threo* acid and a different retention time from the synthetic *erythro* isomer. Finally the *threo* acid obtained by ozonolysis was isolated by preparative tlc on Merck Kieselgel PF_{254} using a solvent system of ethanol-water-25% aqueous NH_4OH (10:15:100). Under these conditions the *erythro* isomer gave R_f 0.58, while the *threo* isomer showed a R_f 0.53 by detection with iodine or $KMnO_4$. In a similar manner *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (mp 43–44°; $J_{2,3}$ 4.2 Hz) on ozonolysis gave exclusively *erythro*-2,3-dihydroxy-4-methylpentanoic acid, identified and characterized as described above.

STEREOSPECIFIC SYNTHESES OF *erythro* AND *threo*-2,3-DIHYDROXY-4-METHYLPENTANOIC ACIDS.—1,3-Dibromo-4-methyl-2-pentanone (bp 95–97°/10 mm $n^{25}d=1.5099$) was prepared by bromination of methylisobutyl ketone and then submitted to the Favorsky reaction to give, in 80% yield, *cis*-4-methyl-2-pentanoic acid (**31**) according to the previously described procedure (55). It had the following physical properties: mp 15.5–17.5°; $n^{25}d=1.4420$; δ(CCl_4) 1.20 (3 H, d, J= 7 Hz), 1.28 (3H, d, J=7 Hz), 3.78 (1 H, m), 5.78 (1 H, d, J=11.5 Hz), 6.22 (1 H, dd, J=11.5, 9.5 Hz), 11.91 (1 H). *Cis*-4-methyl-2-pentanoic acid (**31**) was stereospecifically *cis* hydroxylated as follows. To 4.45 g of the *cis* acid in a solution of 150 ml of water, 100 ml of dioxane and 5 ml of a 1% solution of OSO_4 in an ice bath, was added 3 g of $AgClO_3$ in ¼ g portions over a period of 3 days, in the dark. The silver chloride precipitate was removed by filtration, and it was washed with dilute HCl. Hydrogen sulfide was bubbled through the filtrate for 0.5 hr, then the solution was neutralized with a sodium hydroxide solution. Evaporation of the solution gave a heavy syrup which was crystallized from ethyl acetate to give *erythro*-2,3-dihydroxy-4-methylpentanoic acid (**29**): mp 127°; Calc. for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.68; H, 8.00; νKBr 3250, 1680, 1390, 1355, 1290, 1230, 1130, 1095, 1020, 920, 840, 760 cm^{-1}; δ(D_2O): 0.94 (6 H, d, J=6.75 Hz), 1.92 (1 H, m), 3.53 (1 H, dd, J=6, 5.5 Hz), 4.29 (1 H, d, J=5.5 Hz).

Threo-2,3-Dihydroxy-4-methylpentanoic acid (**28**) was prepared as follows. Malonic acid (83.2 g) was dissolved in a solution of 300 ml of pyridine and 4 ml of piperidine, then 57.6 g of isobutyraldehyde was added and the solution was refluxed for 24 hr. Removal of the water and pyridine at atmospheric pressure gave a crude product which was distilled to give 47.5 g (52%) of *trans*-4-methylpentanoic acid (30), bp 54–55°/0.15 mm, rpt. bp 100°/6 min (56); n_d 1.4481, rpt n_d 1.4487 (56); δ($CDCl_3$): 1.06 (6 H, d, J=7 Hz), 2.45 (1 H, m), 5.74 (1 H, dd, J= 15.5, 1.5 Hz), 7.00 (1 H, dd, J=15.5, 7 Hz), 12.45 (1 H). *Cis* hydroxylation of *trans*-4-methylpentanoic acid (4.4 g), as described above, gave 3.1 g (55%) of *threo*-2,3-dihydroxy-4-methylpentanoic acid (**28**): mp 111–112° (from ethyl acetate); Calc. for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.80; H, 8.05; νKBr 3250–3400, 1680, 1370, 1275, 1240, 1130, 1080, 1050, 1030, 915, 840, 785 cm^{-1}; δ(D_2O): 0.98 (6 H, d, J=6.5 Hz), 1.83 (1 H, m), 3.54 (1 H, dd, J=9.2 Hz), 4.42 (1 H, d, J=2 Hz).

SYNTHESES OF *cis* AND *trans*-2-METHYL, 2-ETHYL, 2-n-PROPYL-3-HYDROXY-5-BROMO-2,3-DIHYDROBENZOFURAN.—Following the procedure previously described for the synthesis of 2'-hydroxy-2,5'-dibromo-3-methylbutyrophenone (37), 2'-hydroxy-2,5'-dibromopropiophenone 2'-hydroxy-2,5'-dibromobutyrophenone and 2'-hydroxy-2,5'-dibromovalerophenone were prepared.

2'-Hydroxy-2,5'dibromopropiophenone.—It exhibited the following physical properties: mp 98–99° (from EtOH); Calc. for $C_9H_8O_2Br_2$: C, 35.10; H, 2.62; Br, 51.89. Found: C, 35.14; H, 2.79; Br, 52.00; δ(CCl_4): 1.87 (3 H, d, J=6.5 Hz), 5.16 (1 H, q, 5.16 Hz), 6.85 (1 H, d, J=9 Hz), 7.49 (1 H, dd, J=9.2 Hz), 7.82 (1 H, d, J=2 Hz).

2'-Hydroxy-2,5'dibromobutyrophenone.—It exhibited the following properties: mp 56–57° (from EtOH); Calc. for $C_{10}H_{10}O_2Br_2$: C, 37.30; H, 3.13; Br, 49.63. Found: C, 36.91; H, 2.94; Br, 49.20; δ(CCl_4): 1.10 (3 H, t, J=7.5 Hz), 2.12 (2 H, m), 5.92 (1 H, t, J=7 Hz), 6.84 (1 H, d, J=9 Hz), 7.48 (1 H, dd, J=9.2 Hz), 7.79 (1 H, d, J=2 Hz).

2'Hydroxy-,2'5dibromovalerophenone.—The following properties were observed: mp 55–56° (from EtOH); Calc. for $C_{11}H_{12}O_2Br_2$: C, 39.31; H, 3.60; Br, 47.56. Found: C, 38.84; H, 3.87; Br, 46.94; δ(CCl_4): 1.02 (3 H, t, J=7.5 Hz), 1.50 (2 H, m), 2.08 (2 H, m), 5.98 (1 H, t, J=7 Hz), 6.85 (1 H, d, J=9 Hz), 7.48 (1 H, dd, J=9.2 Hz), 7.78 (1 H, d, J=2 Hz).

Following the procedures previously described (37) for the preparation of *cis* and *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran, respectively, *cis* and *trans* 2-methyl, 2-ethyl and 2-n-propyl-3-hydroxy-2,3-dihydrobenzofurans were prepared. The compounds were only characterized by their ^1H nmr spectra, which are given below.

trans-2-Methyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**23**).—δ(CCl_4, D_2O): 1.24 (3 H, d, J= 6.5 Hz), 4.40 (1 H, m), 4.59 (1 H, d, J=3.5 Hz), 6.57 (1 H, d, J=8.5 Hz), 7.21 (1H, dd, J=8.5, 2 Hz), 7.30 (1 H, d, J=2 Hz).

cis-2-Methyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**22**).—δ(CCl_4, D_2O): 1.35 (3 H, d, J=6.5 Hz), 4.43 (1 H, m), 4.68 (1 H, d, J=5.5 Hz), 6.55 (1 H, d, J=8.5 Hz), 7.19 (1 H, dd, J=8.5, 2 Hz), 7.30 (1 H, d, J=2 Hz).

trans-2-Ethyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**25**).—δ(CCl_4, D_2O): 1.05 (3 H, t, J=7 Hz), 1.57 (2 H, m), 4.29 (1 H, m), 4.80 (1 H, d, J=3.5 Hz); 6.59 (1 H, d, J=9 Hz), 7.22 (1 H, dd, J=9.2 Hz), 7.35 (1 H, d, J=2 Hz).

cis-2-Ethyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**24**).—δ(CCl_4, D_2O): 1.08 (3 H, t, J=7 Hz), 1.5–1.8 (2 H, m), 4.20 (1 H, m), 4.76 (1 H, d, J=6 Hz), 6.56 (1 H, d, J=9 Hz), 7.20 (1 H, dd, J=9,2 Hz), 7.32 (1 H, d, J=2 Hz).

trans-2-Propyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**27**).—δ(CCl_4, D_2O): 1.12 (3 H, t, J=7 Hz), 1.51 (4 H, m), 4.32 (1 H, m), 4.74 (1 H, d, J=3.7 Hz), 6.58 (1 H, d, J=8.5 Hz), 7.22 (1 H, dd, J=8.5, 2 Hz), 7.32 (1 H, d, J=2 Hz).

cis-2-Propyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**26**).—δ(CCl_4, D_2O): 1.13 (3 H, t, J=7 Hz), 1.51 (4 H, m), 4.31 (1 H, m), 4.82 (1 H, d, J=5.5 Hz), 6.58 (1 H, d, J=8.5 Hz), 7.23 (1 H, dd, J=8.5, 2 Hz), 7.32 (1 H, d, J=2 Hz).

ACKNOWLEDGMENTS

We express our sincere appreciation to the National Cancer Institute, NIH, for support of this work (CA-18819). We also thank Dr. C. Dickinson, Dr. M. Gordon, and Ms. S. Bonetti for performing various individual experiments related to this work. The FOFC tables have been deposited with the Editor.

Received 16 October 1978.

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