previously with 10^5 L1210 tumor cells. Cells were removed by centrifugation. The enzyme assay was incubated for 30 min at 37 °C and the reaction stopped with 10% phosphotungstic acid (PTA) in 0.5 N HCl. The precipitate was washed with 10% trichloroacetic acid (TCA) and once with ethanol/ether, 2:1. The precipitate was dissolved in 200 μL of 1 N NaOH and neutralized with 1 N HCl, and the solution was counted in 10 mL of scintillation fluid (Liquiscint 2, National Diagnostics, Somerville, NJ) by using a β-scintillation counter (efficiency 33% for tritium). All other chemicals were purchased from either Fisher Scientific, or Sigma. All assays were performed in duplicate. The enzyme kinetic calculations were performed by using a software program by Dr. William Greco.[29] Data were graphed as δ vs. [S], v vs. [I], $1/v$ vs. $1/[S]$, and $1/v$ vs. [I] and the kinetic constants obtained from these plots.

Inhibition of Tumor Cell Growth in Vitro. Compounds were evaluated for their ability to inhibit growth of L1210 leukemia, human B-lymphoblastic leukemia (WI-L2), and human T-lymphoblastic leukemia (CEM) according to our previous procedure.[30]

In Vitro Antiviral Evaluations. Glycopyranosyl phosphonates and their nucleotide sugar analogues were evaluated for their ability to inhibit virus-induced cytopathic effect (CPE) produced by six viruses. Viruses used in this evaluation included parainfluenza virus type 3 (para 3) strain C243, herpes simplex virus type 2 (HSV-2) strain MS, adenovirus type 2, rhinovirus, influenza virus, and visna virus. Antiviral experiments were performed according to our previous procedures.[30]

Acknowledgment. We thank Marilyn Hillman for excellent technical assistance.

Supplementary Material Available: Analytical data for compounds **2**, **5**, **8–17**, **20**, **23**, and **26** (Table 15) and ^{1}H and ^{13}C NMR spectral data for glycopyranosyl methylenediphosphonates (Table 25) (3 pages). Ordering information is given on any current masthead page.

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Effects of Derivatives of Kahweol and Cafestol on the Activity of Glutathione S-Transferase in Mice

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The coffee constituents cafestol and kahweol are inducers of the activity of the detoxifying enzyme, glutathione S-transferase in laboratory animals. The two active functional groups, furan and glycol, on opposite ends of the diterpene nucleus of these two compounds have been modified. The resulting derivatives were evaluated for their ability to induce glutathione S-transferase activity in different tissues of the ICR/Ha mice. Derivatives of both cafestol and kahweol, which were the products of the modifications on the glycol function, retained much of the inducing properties of the parent compounds in the liver and mucosa of the small bowel. The effects of these compounds on the tissue acid-soluble sulfhydryl level, in general, were similar to those of the parent compounds. Some derivatives of kahweol, however, appeared to have lost their ability to induce increased levels of sulfhydryls in the liver. Catalytic hydrogenation of the furan moiety gave two products, the dihydro and tetrahydro derivatives. Both compounds and their corresponding acetates lost their effectiveness as inducers of glutathione S-transferase activity. In contrast, these compounds still retained their ability to induce increased levels of acid-soluble sulfhydryls in both the liver and the mucosa of the small bowel. These findings indicate that the furan moiety of cafestol and kahweol is vital to their biological activity as inducers of increased glutathione S-transferase activity.

Glutathione S-transferase (GST) is a major detoxifying enzyme system that catalyzes the binding of a variety of electrophiles, including the reactive forms of chemical carcinogens, to glutathione.[1,2] In most instances this conjugation results in rendering these substances to be less harmful and facilitates their excretion. Thus the enhancement of the activity of this enzyme system potentially could increase the capacity of the organism to withstand the neoplastic effects of chemical carcinogens. Since glutathione is a mandatory substrate in the conjugation of xenobiotics catalyzed by this enzyme system, the concentration of this compound in the tissues may be vital in the overall detoxifying process.

Green coffee beans fed in the diet were found to induce increased GST activity and tissue sulfhydryl (SH) level in the mucosa of the small intestine and in the liver of rats and mice. Using the induction of GST activity as an assay method, we followed the enzyme-inducing activity of the extracts of green coffee beans and isolated from the petroleum ether fraction two diterpene esters, kahweol palmitate and cafestol palmitate. These two natural products are potent inducers of the GST enzyme system and are good inducers of tissue SH level.[3]

The parent compounds of these two esters are similar diterpenes differing from each other by one double bond on the A ring of the diterpene nucleus.[4,5] Kahweol, IIa, which has the Δ^1 double bond, is a better inducer of the GST activity than cafestol. This difference in activity as a result of small change in the diterpene structure suggests the possibility of producing a more potent inducer by modifying the structure of the parent compound. Besides the double bond on kahweol (IIa) there are two functional groups on opposite ends of the diterpene nucleus of both cafestol (Ia) and kahweol (IIa). A furan ring is attached to the A ring at C3 and C4 while a glycol function is at C16 and C17. To determine the contribution of these functional groups to the inducing potential of increased GST activity, studies of structure activity relationships were

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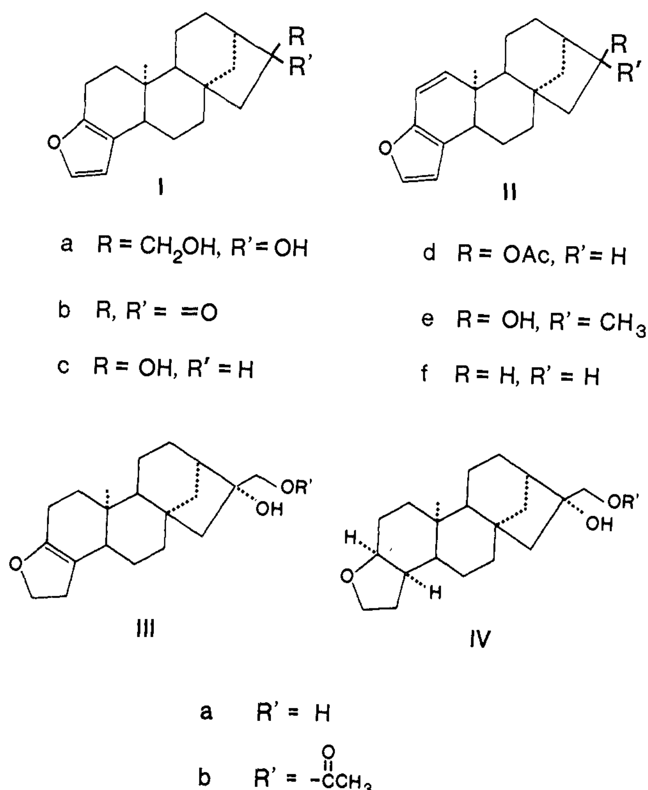
Chart I



carried out on the various derivatives of kahweol and cafestol.

Chemistry

Modifications on Ia and IIa (Chart I) were carried out such that the basic diterpene nucleus remains unchanged. The first set of derivatives (b–f) are those resulting from the modification of the glycol group at C16–C17 and the second set of derivatives are those from the modification of the furan moiety. The reaction conditions for both series I and II were identical. For the preparation of the ketones Ib and IIb, a mixture of Ia and IIa were used. Other conversions were performed on pure compounds from either series I or series II.

The ketones Ib and IIb were obtained by the lead tetraacetate oxidation of a mixture of Ia and IIa in pyridine. The use of pyridine as the reaction solvent is preferred over benzene, which was used in the procedure of Wettstein et al.[6] When the usual solvent, benzene, was used, the reaction was very difficult to control and the products of oxidation contained numerous side products. In pyridine, however, the glycol was oxidized to the ketone with very little reaction side products. The pure ketones Ib and IIb were separated by preparative LC on a recycling mode.

The ketone (Ib or IIb) was reduced by sodium borohydride to give the alcohol Ic (IIc) in good yield. The reduction gave predominantly the endo alcohol with the hydroxy group at the β face of the molecule.[7] the exo proton at C-16 was confirmed by the NMR resonances at 4.35 ppm (m, 1 H).[8] Recrystallization from hexane eliminated the presence of the trace of the other isomer. The corresponding acetate, Id (IId), was obtained by the reaction of acetyl chloride with Ic (IIc) in pyridine.

Compound Ie (IIe) was synthesized by the reaction of methylmagnesium iodide with Ib (IIb) in ether. The stereochemistry at C16 of Ie and IIe has not been rigorously determined. The normal Grignard reaction on a bicyclic ketone of the type like Ib (IIb) is expected to give a final product with the hydroxy group located at the β face of the molecule (endo isomer).[9]

The compound If (IIf) was obtained by the Wolff–Kischner reduction of Ib.

The furan moiety of the diterpenes was modified by catalytic hydrogenation. Cafestol gave two products, depending on the hydrogen pressure and the amount of catalyst used. At pressure greater than 30 psi 2 equiv mol of hydrogen were consumed and tetrahydrocafestol was obtained.[10] When the hydrogen pressure was lowered to 10 psi and the amount of catalyst reduced, the product of hydrogenation was mainly dihydrocafestol. The absence of the NMR resonances at 6.3 and 7.28 ppm of IIIa indicate the addition of two hydrogens at C18 and C19. The stereochemistry at the ring juncture of IVa remains to be determined.

Table I. Effects of Derivatives of Cafestol and Kahweol on the GST Activity in the Cytosols of Liver and Small Bowel Mucosa of ICR/Ha Mice

	liver		small bowel mucosa	
compd	specific activity[a,b]	ratio[c]	specific activity[a,b]	ratio[c]
Ia	3.35 ± 0.22	2.0 ± 0.2	1.36 ± 0.03	3.8 ± 0.1
Ib	2.85 ± 0.07	1.7 ± 0.1	2.06 ± 0.05	5.7 ± 0.2
Ic	2.54 ± 0.12	1.5 ± 0.1	1.52 ± 0.14	4.2 ± 0.4
Id	2.24 ± 0.14	1.3 ± 0.1	1.83 ± 0.07	5.1 ± 0.2
Ie	2.26 ± 0.09	1.4 ± 0.1	1.30 ± 0.06	3.6 ± 0.2
If	2.62 ± 0.13	1.6 ± 0.1	0.95 ± 0.04	2.6 ± 0.1
control	1.67 ± 0.07		0.36 ± 0.01	
IIa	4.30 ± 0.17	3.0 ± 0.1	2.21 ± 0.22	7.3 ± 0.8
IIb	3.67 ± 0.10	2.6 ± 0.1	1.84 ± 0.20	6.1 ± 0.7
IIc	3.57 ± 0.19	2.5 ± 0.1	1.72 ± 0.04	5.7 ± 0.2
IId	3.68 ± 0.19	2.6 ± 0.2	2.06 ± 0.24	6.9 ± 0.8
IIe	2.59 ± 0.15	1.8 ± 0.1	1.34 ± 0.02	4.5 ± 0.1
IIf	3.06 ± 0.02	2.1 ± 0.1	1.18 ± 0.08	3.9 ± 0.3
control	1.44 ± 0.03		0.30 ± 0.01	

[a] GST activity was determined spectrophotometrically at 30 °C with 1-chloro-2,4-dinitrobenzene as the substrate. Specific activities are expressed as [μmol min^{-1} (mg of protein)$^{-1}$] ± SD. [b] All values (Ia–IIF) – $P < 0.005$. [c] The ratio is tested compound/control.

Biological Activity

The effects of the derivatives of Ia and IIa on the increased activity of the detoxifying enzyme system, GST, in various tissues of ICR/Ha mice were determined according to the method of Habig et al.[11] The acid-soluble sulfhydryl level in the liver and small bowel mucosa was determined according to the method of Ellman.[12] The test compounds were dissolved in cottonseed oil with 10% ethanol and were administered by po intubation.

The activity (test/control) of Ia and its derivatives (Ib–f) in inducing increased GST in the liver ranged from 2.0 to 1.3 (Table I). Thus all the derivatives of Ia modified at the glycol moiety showed similar inducing activity in this tissue. In the kahweol series, modification of the glycol function retained most of the activity to induce increased GST in the liver. Compound IIe showed the lowest ac-

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Table II. Effects of Derivatives of Cafestol and Kahweol on the Tissue Acid-Soluble Sulfhydryl Levels of Liver and Small Bowel Mucosa of ICR/Ha Mice

	liver		small bowel mucosa	
compd	SH level[a]	ratio[c]	SH level[a]	ratio[c]
Ia	14.9 ± 0.3[b]	2.0 ± 0.1	7.9 ± 0.1[b]	1.5 ± 0.1
Ib	13.3 ± 0.2[b]	1.8 ± 0.1	10.3 ± 0.3[b]	1.9 ± 0.1
Ic	12.8 ± 0.1[b]	1.7 ± 0.1	11.9 ± 0.1[b]	2.2 ± 0.1
Id	13.3 ± 0.5[b]	1.8 ± 0.1	8.3 ± 0.4[b]	1.5 ± 0.1
Ie	16.3 ± 0.5[b]	2.1 ± 0.1	8.6 ± 0.1[b]	1.6 ± 0.1
If	13.0 ± 0.3[b]	1.7 ± 0.1	8.4 ± 0.2[b]	1.6 ± 0.1
control	7.6 ± 0.2		5.4 ± 0.1	
IIa	13.0 ± 0.4[b]	1.5 ± 0.1	13.1 ± 0.2[b]	2.1 ± 0.1
IIb	11.8 ± 0.5[b]	1.3 ± 0.1	10.4 ± 0.2[b]	1.6 ± 0.1
IIc	9.5 ± 0.3	1.1 ± 0.1	10.9 ± 0.3[b]	1.7 ± 0.1
IId	10.1 ± 0.2	1.1 ± 0.1	10.8 ± 0.2[b]	1.7 ± 0.1
IIe	8.3 ± 0.3	0.9 ± 0.1	7.7 ± 0.1[b]	1.2 ± 0.1
IIf	9.5 ± 0.3	1.1 ± 0.1	7.8 ± 0.3[b]	1.2 ± 0.1
control	8.9 ± 0.3		6.3 ± 0.1	

[a] The SH level was determined spectrophotometrically according to the method of Ellman.[9] Tissue SH levels are expressed as (μmol of SH/g of tissue) ± SD. [b] $P < 0.005$ [c] The ratio is test compound/control.

tivity, which is approximately 60% of the parent compound IIa.

In the mucosa of the small bowel the inducing activities of the cafestol derivatives on GST activity were higher than those in the liver. The ketone Ib gave the highest relative increase in GST activity (5.7), while the hydrocarbon If gave the lowest increase (2.6). The activity of Ia (3.8) falls in the middle as an inducer of GST (Table I). All the kahweol derivatives were highly effective as inducers of GST activity in the small bowel mucosa. Kahweol itself was the most active; the hydrocarbon IIf had the lowest activity which was 50% that of the parent compound (Table I).

In addition to the induction of the activity of the enzyme system GST, Ia was found to induce increased tissue sulfhydryl level.[3] Modifications of the glycol function of Ia did not alter appreciably the ability of the resulting derivatives to induce increased tissue sulfhydryl level in the liver (Table II).

In contrast to the cafestol series, the kahweol derivatives IIc, IIe, and IIf apparently lost their ability to induce increased level of acid-soluble sulfhydryl in the liver. Compound IId retained partial activity as an inducer while IIb was 70% as effective as the parent compound (Table II).

As an inducer of increased SH level in the small bowel mucosa, Ib and Ic were more effective than other derivatives that were as effective as cafestol, the parent compound. All the kahweol (IIa–f) derivatives were also active as inducers of increased SH content in this tissue. In this case the parent compound was the most active compound, the derivatives IIb, IIc, and IId were over 60% as active, while compounds IIe and IIf were only 20% as active. In the forestomach and lung the increase in GST activity by these compounds was much lower than in the small bowel mucosa (Table III). The highest response to induction of GST activity in the forestomach was for the parent compound, kahweol, IIa. Conversion of IIa to the hydrocarbon IIf resulted in a compound that did not effect any increase of GST activity. Other derivatives of IIa gave moderate induction while administering the compounds in the cafestol series resulted only in slight or no increases above the control level. In the lung, none of the compounds tested showed any appreciable effect on GST activity.

Catalytic hydrogenation of cafestol and its acetate gave two products from each compound. One was a dihydro derivative IIIa (IIIb) and the other was a tetrahydro derivative IVa (IVb). The activity of IIIa (IIIb) and IVa (IVb) to induce increased enzyme activity of GST was determined in the liver and small bowel mucosa. With the exception of IVa, which showed slight activity, none of the other hydrogenated derivatives showed any significant inducing activity. The reference compound, cafestol acetate, in the experiment gave the normal activity of 2.74 and 7.5 respectively for the liver and small bowel mucosa (Table IV)[3]. These results indicate an elimination of the inducing activity of cafestol when the furan moiety of the molecule is saturated.

The effects of these hydrogenated derivatives on the tissue SH level, however, were significant. While the tissue SH level in the small bowel mucosa was induced at a lower level by the hydrogenated products than by the reference compound, cafestol acetate, the tissue SH level in the liver was induced to about the same level by these derivatives as by cafestol acetate. Only compound IIIa in this series showed lower activity than cafestol acetate (Table V).

Table III. Effects of Derivatives of Cafestol and Kahweol on the GST Activity in the Cytosol of the Forestomach and Lung of ICR/Ha Mice

	forestomach		lung	
	specific activity[a]	ratio[b]	specific activity[a]	ratio[b]
Ia	0.91 ± 0.01	1.2 ± 0.03	0.26 ± 0.02	1.0 ± 0.1
Ib	0.88 ± 0.02	1.2 ± 0.03	0.29 ± 0.01	1.1 ± 0.1
Ic	0.91 ± 0.02	1.2 ± 0.04	0.20 ± 0.01	0.8 ± 0.1
Id	0.83 ± 0.02	1.1 ± 0.04	0.29 ± 0.01	1.1 ± 0.1
Ie	0.76 ± 0.02	1.0 ± 0.04	0.27 ± 0.01	1.0 ± 0.1
If	0.85 ± 0.01	1.1 ± 0.03	0.33 ± 0.02	1.3 ± 0.1
control	0.75 ± 0.02		0.26 ± 0.01	
IIa	1.40 ± 0.12	2.1 ± 0.2	0.29 ± 0.01	1.0 ± 0.1
IIb	1.05 ± 0.04	1.6 ± 0.1	0.28 ± 0.01	1.0 ± 0.1
IIc	1.01 ± 0.04	1.5 ± 0.1	0.23 ± 0.01	0.8 ± 0.1
IId	0.94 ± 0.02	1.4 ± 0.1	0.30 ± 0.02	1.0 ± 0.1
IIe	0.92 ± 0.02	1.4 ± 0.1	0.24 ± 0.01	0.8 ± 0.1
IIf	0.63 ± 0.03	0.9 ± 0.1	0.36 ± 0.01	1.2 ± 0.1
control	0.66 ± 0.02		0.29 ± 0.01	

[a] GST activity was determined spectrophotometrically at 30 °C with 1-chloro-2,4-dinitrobenzene as the substrate. Specific activities are expressed as [μmol min^{-1} (mg of protein)$^{-1}$] ± SD. [b] The ratio is test compound/control.

Table IV. Effects of Dihydro- and Tetrahydrocafestol and Their Monoacetates on the GST Activity in the Cytosols of the Liver and Small Bowel Mucosa of ICR/Ha Mice

	liver		small bowel mucosa	
	specific activity[a]	ratio[c]	specific activity[a]	ratio[c]
IIIa	1.22 ± 0.05	1.04 ± 0.05	0.42 ± 0.02	1.1 ± 0.1
IIIb	1.27 ± 0.06	1.08 ± 0.05	0.41 ± 0.02	1.0 ± 0.1
IVa	1.48 ± 0.03[b]	1.25 ± 0.04	0.54 ± 0.02[b]	1.4 ± 0.1
IVb	1.25 ± 0.04	1.06 ± 0.04	0.41 ± 0.01	1.0 ± 0.1
cafestol acetate	3.24 ± 0.31[b]	2.74 ± 0.27	3.00 ± 0.20[b]	7.5 ± 0.6
control	1.18 ± 0.30		0.40 ± 0.02	

[a] GST activity was determined spectrophotometrically at 30 °C with 1-chloro-2,4-dinitrobenzene as the substrate. Specific activities are expressed as [μmol min^{-1} (mg of protein)$^{-1}$] ± SD. [b] $P <$ 0.005. [c] The ratio is test compound/control.

Discussion

The results of this investigation indicate that modification of the glycol function of the diterpenes Ia and IIa does not alter appreciably their biological activity to induce increased activity of the detoxifying enzyme system, GST. The hydrogenation of the furan moiety, however, gives a dihydro and a tetrahydro compound that are inactive as inducers of GST. This latter finding indicates that the

Table V. Effects of Dihydro- and Tetrahydrocafestol and Their Monoacetates on the Tissue Acid-Soluble Sulfhydryl Levels of Liver and Small Bowel Mucosa of ICR/Ha Mice

	liver		small bowel mucosa	
	SH level[a,b]	ratio[c]	SH level[a,b]	ratio[c]
IIIa	11.9 ± 0.3	1.18 ± 0.05	6.5 ± 0.1	1.1 ± 0.03
IIIb	15.6 ± 0.7	1.55 ± 0.09	7.1 ± 0.2	1.2 ± 0.04
IVa	14.5 ± 0.1	1.44 ± 0.05	7.4 ± 0.1	1.2 ± 0.03
IVb	14.3 ± 0.3	1.42 ± 0.06	8.2 ± 0.2	1.4 ± 0.07
cafestol acetate	14.3 ± 0.4	1.41 ± 0.06	12.0 ± 0.4	2.0 ± 0.07
control	10.1 ± 0.4		6.0 ± 0.1	

[a] The SH level was determined spectrophotometrically according to the method of Ellman.[9] Tissue SH levels are expressed as (μmol of SH/g of tissue) ± SD. [b] All values (IIIa–Caf Ac) – $P < 0.005$. [c] The ratio is test compound/control.

furan moiety is vital to the biological activity of Ia as an enzyme inducer. The corresponding di- or tetrahydro compounds of IIa have not been investigated. Because of the similarity of the two compounds, Ia and IIa, it is anticipated that the inducing activity of the derivatives of IIa will be reduced similarly as a result of saturation at the furan ring.

The palmitates of Ia and IIa are naturally occurring esters that induce increased activity of the enzyme system GST as well as an increase in the tissue sulfhydryl level. These derivatives, when administered to rats, inhibit mammary tumor formation induced by the carcinogen 7,12-dimethylbenz[*a*]anthracene.[13]

These findings support the observation that inducers of the detoxifying enzyme GST are inhibitors of carcinogenesis.[14] The identification of the active site on the diterpene nucleus that is responsible for the enzyme-inducing activity may lead to the identification of the active moiety on the molecule that is responsible for the inhibition of carcinogenesis.

In contrast to the reduction of inducing activity of GST, the partially or totally saturated furan moiety retains part (in the small bowel mucosa) or all of the activity (in the liver) in the induction of increased tissue SH level. This separation of the inducing activities of the enzyme and increased SH level may be used as a probe to study the relative effects of the activity of the GST vs. the cofactor glutathione in the inhibition of carcinogenesis.

Experimental Section

Melting points were determined on a Fisher-John Mel-temp apparatus and were uncorrected. Infrared spectra were recorded on a Beckman Acculab V spectrophotometer. NMR spectra were determined with a Brücker WM 250 spectrometer with tetramethylsilane as internal standard. Mass spectra were taken on a LKB 9000 GC–MS spectrometer at the Gortner Biochemistry Laboratory of the University of Minnesota, St. Paul, MN. Elemental analyses on chemical compounds were done by Galbraith Laboratories, Inc. (P. O. Box 4187, Knoxville, IN 37912); results were within 0.4% of theoretical values except where indicated.

16-Oxocafestol (Ib) and 16-Oxokahweol (IIb). The ketones Ib and IIb were prepared by the lead tetraacetate oxidation of a mixture of Ia and IIa according to the procedure of Wettstein et al.[4,6,15,16] with the following modifications. A solution containing 10 g (0.032 mol) of Ia and IIa mixture in 200 mL of pyridine was cooled to 0 °C in an ice bath. To this solution was added, in small portions, 14 g (0.032 mol) of lead tetraacetate in 15 min. The reaction mixture was then poured into ice immediately and the products were extracted with ethyl acetate. The organic layer was washed with 6 N HCl, saturated $NaHCO_3$, and water. The organic solution was dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to give 9.4 g (100%) of crude product. Separation of the mixture by preparative LC using two silver nitrate impregnated silver cartridges (hexane–ethyl acetate, 5:1, v/v; flow rate 200 mL/min)[17] yielded pure samples of Ib, 2.5 g (28% of original mixture), mp 167.5–169 °C (lit.[15,16] mp 176–178 °C); IIb, 3.2 g (35% of original mixture), mp 183.5–184 °C.

Ib: IR (CCl_4) 1745 cm^{-1} (C=O); MS (20 eV, *m/z* (relative intensity)), 284 (100), 269 (17), 148 (9.9); NMR ($CDCl_3$) δ 0.914 (s, 3 H), 6.24 (d, 1 H), 7.26 (d, 1 H). Anal. ($C_{19}H_{24}O_2$) C, H; C: calcd, 80.24; found, 79.79.

IIb: IR (CCl_4) 3050 (C=CH), 1745 cm^{-1} (C=O); MS (20 eV, *m/z* (relative intensity)), 282 (100), 267 (20.7), 145 (68.3); NMR ($CDCl_3$) δ 0.99 (s, 3 H), 5.96 (d, 1 H), 6.27 (d, 1 H), 6.31 (s, 1 H), 7.28 (s, 1 H). Anal. ($C_{19}H_{22}O_2$) C, H.

16-Deoxy-16-norcafestol (Ic) and 16-Deoxy-16-norkahweol (IIc). A solution containing 1.0 g (3.5 mmol) of the ketone (Ib or IIb) in 50 mL of absolute methanol and 20 mL of tetrahydrofuran was cooled in an ice bath. To this solution was added 250 mg (6.6 mmol) of sodium borohydride in small portions. The reaction mixture was allowed to warm up to room temperature. The content of the reaction was poured into ice water and was neutralized with 6 N hydrochloric acid. The product was extracted into ethyl acetate. Crystallization from hexane yielded 910 mg (91%) of Ic: mp 90–91 °C; IR (CCl_4) 3620 (free OH), 3380 cm^{-1} (bonded OH); MS (20 eV, *m/z* (relative intensity)), 286 (100), 271 (7.5), 148 (13.9); NMR ($CDCl_3$) δ 0.847 (s, 3 H), 4.35 (m, 1 H). Anal. ($C_{19}H_{26}O_2$) C, H; H: calcd, 9.15; found, 8.60.

IIc: 740 mg (74%); mp 103–105 °C; IR (CCl_4) 3620 (free OH), 3480 (bonded OH), 3040 cm^{-1} (vinyl C–H); MS (20 eV, *m/z* (relative intensity)), 284 (60.8), 269 (3.8), 146 (100); NMR (CCl_4) δ 0.992 (s, 3 H), 5.97 (d, 1 H), 6.29 (d, 1 H), 6.31 (s, 1 H), 7.28 (s, 1 H). Anal. ($C_{19}H_{24}O_2$) C, H.

16-Deoxy-16-norcafestol Acetate (Id) and 16-Deoxy-16-norkahweol Acetate (IId). To a cooled (O °C) solution containing 1.0 g (3.2 mmol) Ic or IIc in 10 mL of pyridine was added dropwise 0.3 mL (3.5 mmol) of acetyl chloride with stirring. The mixture was then allowed to warm up to room temperature and stirring was continued overnight. The reaction mixture was poured onto ice and the aqueous solution extracted three times with 15 mL of ether. The ethereal extracts were combined and washed with 6 N HCl until all the pyridine was neutralized. The organic layer was washed with saturated $NaHCO_3$ solution and water. The solvent was removed in vaccuo. The acetate was crystallized from EtOAc–hexane. Id: yield 1.08 g (95%), mp 157–158 °C; IR (CCl_4) 1770 cm^{-1} (C=O); MS (20 eV, *m/z* (relative intensity)) 328 (100), 268 (11.7), 253 (18.7), 239 (27.4), 148 (22.2); NMR ($CDCl_3$) δ 0.84 (s, 3 H), 2.07 (s, 3 H), 5.06 (m, 1 H), 6.21 (d, 1 H, J = 1.8 Hz).

IId: yield 1.02 g (90%), mp 142–143 °C; IR(CCl_4) 3040 (vinyl C–H), 1770 cm^{-1} (C=O); MS (20 eV, *m/z* relative intensity)), 326 (49.9), 266 (13.2), 251 (19.6), 146 (100); NMR ($CDCl_3$) δ 0.98 (s, 3 H), 208 (s, 3 H), 5.07 (m, 1 H), 5.94 (d, 1 H, J = 10.02 Hz), 6.25 (d, 1 H, J = 10.11 Hz), 6.31 (s, 1 H), 7.28 (s, 1 H).

16-Methylcafestadien-16-ol (Ie) and 16-Methylkahweadien-16-ol (IIe). To a solution containing 284 mg (1.0 mmol) of the ketone (Ib or IIb) in 20 mL of tetrahydrofuran was added dropwise a 2 M ether solution (0.8 mL) of methylmagnesium iodide at room temperature. The reaction mixture was allowed to stand at room temperature with stirring for 2 h. The content of the reaction was then poured into ice water and the product was extracted with ethyl acetate. Purification by preparative LC gave 140 mg (47%) of Ie and 120 mg (40%) of IIe, respectively.

Ie: mp 168.5–170 °C; IR (CCl_4) 3610 (free OH), 3480 cm^{-1} (bonded OH); MS (20 eV, *m/z* (relative intensity)) 300 (100), 282 (26.6), 267 (16.7), 148 (18.7); NMR ($CDCl_3$) δ 0.84 (s, 3 H), 1.35 (s, 3 H), 6.25 (d, 1 H, J = 1.6 Hz), 7.24 (d, 1 H, J = 1.6 Hz).

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IIe: mp 95–96 °C; IR (CCl_4) 3610 (free OH), 3480 (bonded OH), 3050 cm^{-1} (vinyl C–H); MS (20 eV, *m/z* (relative intensity)), 298 (49.2), 280 (24.1), 265 (11.8), 146 (100); NMR ($CDCl_3$) δ 0.99 (s, 3 H), 1.36 (s, 3 H), 5.97 (d, 1 H, J = 9.97 Hz), 6.25 (d, 1 H, J = 10.07 Hz), 6.30 (d, 1 H, J = 2.66 Hz), 7.27 (d, 1 J = 2.66 Hz).

Cafestadiene (If) and Kahweadiene (IIf). A solution containing 0.5 g (1.8 mmol) of Ib (IIb) in 40 mL of diethylene glycol and 3.4 mL of hydrazine was refluxed for 30 min. To this reaction mixture was added 0.5 g of potassium hydroxide and refluxing was continued for 1 h. The condenser was then removed and the water–diethylene glycol mixture was evaporated until the pot temperature reached 190 °C. The condenser was replaced and refluxing was continued for an additional 2.5 h. The reaction mixture was cooled and poured into ice water. The product was collected by filtration.

If: yield 125 mg (25%); after purification by prep LC (prepPak 500/silica, hexane–ethyl acetate, 5:1, v/v) mp 90–91 °C (lit.[16,18] mp 90–93 °C); MS (20 eV, m/z (relative intensity)), 270 (100), 255 (24.9), 241 (10.1), 148 (21.2); NMR ($CDCl_3$) δ 0.82 (s, 3 H), 6.2 (d, 1 H, J = 1.8 Hz), 7.2 (d, 1 H), J = 1.8 Hz).

IIf: yield 100 mg (20%) after purification by prep LC, mp 70–71 °C; MS (20 eV, *m/z* (relative intensity)), 268 (50), 253 (9.3), 239 (5.9), 146 (100); NMR ($CDCl_3$) δ 0.96 (s, 3 H), 5.95 (d, 1 H, J = 10 Hz), 6.24 (d, 1 H, J = 10 Hz), 6.31 (s, 1 H), 7.28 (s, 1 H).

Hydrogenation of Ia and IIa. A solution containing 1.06 g of Ia and IIa in 150 mL of absolute ethanol and 250 mg of palladium on activated carbon was shaken overnight in a Parr hydrogenation apparatus with 10 psi of hydrogen pressure. The catalyst was removed by filtration through a Celite pad. The ethanol was removed in vacuo. The products were separated by prep LC (silica column, hexane–ethyl acetate, 1:2, v/v).

The first fraction was crystallized from ethyl acetate to give 245 mg (23%) of IIIa: mp 186.5–189 °C; MS (20 eV, *m/z* (relative intensity)), 318 (11.8), 300 (39.5), 289 (12.1), 96 (100); NMR ($CDCl_3$) δ 0.98 (s, 3 H), 3.48 (m, 2 H), 3.8 (m, 2 H).

The yield of IVa was 350 mg (33%): mp 154.0–157 °C (Lit.[16] mp 154.5–157 °C); MS (20 eV, *m/z* (relative intensity)), 320 (9.8), 302 (40.5); NMR ($CDCl_3$) δ 0.98 (s, 3 H), 3.48 (m, 2 H), 3.8 (m, 2 H).

To a cooled (O °C) solution containing 500 mg (1.65 mmol) IIIa or IVa in 10 mL of pyridine was added dropwise 0.170 mL (2.0 mmol) of acetyl chloride with stirring. The mixture was then allowed to warm up to room temperature and stirring was continued overnight. The reaction mixture was poured onto ice and the aqueous solution extracted three times with 10 mL of ether. The ethereal extracts were combined and washed with 6 N HCl until all the pyridine was removed. The organic layer was washed with saturated $NaHCO_3$ solution and water. The solvent was removed in vacuo. The acetate was crystallized from EtOAc–hexane.

IIIb: yield 500 mg (90%); mp 159–160 °C; MS (20 eV, *m/z* (relative intensity)), 360 (14.2), 302 (32.9), 287 (23.2); NMR ($CDCl_3$) δ 0.98 (s, 3 H), 2.07 (s, 3 H).

IVb: yield 500 mg (90%), mp 152–154 °C (lit.[19] mp 152–154 °C); MS (20 eV, *m/z* (relative intensity)), 362 (1.1), 344 (4.0), 289 (100), 284 (25.6); NMR ($CDCl_3$) δ 0.98 (s, 3 H), 2.07 (s, 3 H).

Enzyme Assay. Female ICR/Ha mice from Harlan Sprague Dawley Co., Indianapolis, IN, were used for all experiments. Mice were randomized by weight at 6–8 weeks of age, divided into groups of four to five animals each and placed on a semipurified diet ad libitum. The diet consisted of 27% vitamin-free casein, 59% starch, 10% corn oil, 4% salt mix (U.S.P. XIV), and a complete mixture of vitamins (Teklad, Inc., Madison, WI). Four to seven days later the experimental groups were given by po administration the test compound (10 μmol in 0.2 mL of cottonseed oil with 10% ethanol) once daily for 3 consecutive days. The controls received the solvent mixture only. Twenty-four hours after the last administration the mice were killed and the tissues removed. The tissues were homogenized in phosphate buffer, pH 7.5, with a Virtis homogenizer for 45 s.

An aliquot of this homogenate was immediately used for the acid-soluble SH determination. The remaining homogenate was centrifuged at 100000*g* for 1 h. The cytosolic portion thus obtained was used for the GST activity determination.

The activity of cytosolic GST was assayed according to the method of Habig et al.[11] using 1-chloro-2,4-dinitrobenzene as the substrate. The reaction was monitored at 340 nm in a Beckman DU-8 spectrophotometer. Assays were conducted at 30 °C in 0.1 M sodium phosphate buffer, pH 6.5, in the presence of 5 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene. Complete assay mixture without enzyme was used as the control.

Acid-Soluble Sulfhydryl Level. The acid-soluble SH level in tissue homogenates was assayed according to the method of Ellman.[12] Aliquots of tissue homogenates were precipitated with equal volumes of 4% sulfosalicylic acid. The supernatants were assayed for the presence of free SH groups by the addition of 9 × volume of Ellman's reagent [0.1 mM 5,5′-dithiobis(2-nitrobenzoic acid)] in 0.1 M sodium phosphate buffer, pH 8.0. The absorbance was recorded at 412 nm.

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Registry No. Ia, 469-83-0; Ib, 108664-98-8; Ic, 108665-00-5; Id, 108665-02-7; Ie, 108665-04-9; If, 108665-06-1; IIa, 6894-43-5; IIb, 108664-99-9; IIc, 108665-01-6; IId, 108665-03-8; IIe, 108665-05-0; IIf, 108665-07-2; IIIa, 108665-08-3; IIIb, 108665-10-7; IVa, 108665-09-4; IVb, 108665-11-8; GST, 50812-37-8.

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Aldosterone Antagonists. 2. New 7α-(Acetylthio)-15,16-methylene Spirolactones

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Some 15,16-methylene derivatives of the aldosterone antagonist spironolactone (1) were synthesized with the purpose of increasing the antialdosterone potency and reducing the endocrinological effects of this standard compound. By introduction of a 1,2-double bond and a 15β,16β-methylene ring in the spironolactone molecule both goals were achieved. In animal studies mespirenone (13) exhibited a threefold-greater antialdosterone potency and less than 10% of the antiandrogenic activity of spironolactone.

In a previous paper,[1] we described the influence of substituents at the 1,2-position of the steroid framework on the aldosterone antagonistic activity of 6β,7β:15β,16β-dimethylene spirolactones. It was found that the intro-

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