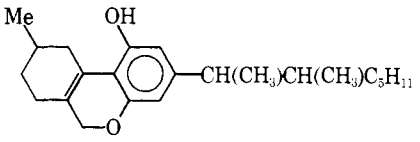
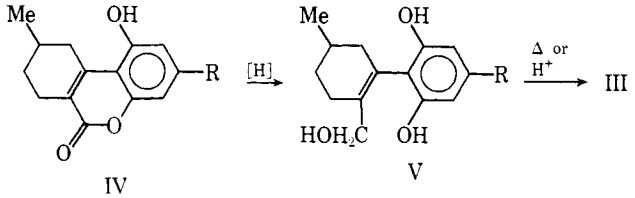
dog. More recently, the 6,6-diethyl analog of the dimethylheptyl compound IIe was shown to be twice as active as synhexyl but to have only 0.004 times the activity of IIe itself.[6] Because of the marked *decrease* in biological activity on *increasing* substitution at this site, we were interested in examining the *lower* homologs, *i.e.*, the 6-mononormethyl and 6,6-dinor compounds. Such cannabinoid compounds have not been described previously. Our investigations regarding the former will be described separately. We wish to report here the synthesis of III, the 6,6-dinor analog of IIe.



III

Acid-catalyzed condensation of ethyl 2-cyclohexanonecarboxylate with 5-(1′,2′-dimethylheptyl)resorcinol gave the coumarin IV.[4] Reduction of IV with lithium aluminum hydride gave the triol V. Cyclization to III can be carried out thermally or, in better yield, by acid catalysis.



IV V

R = $CH(CH_3)CH(CH_3)C_5H_{11}$

Compound III produces CNS depression in rats at doses starting with 10 mg/kg po; it is five times as potent as IId and 1–2.5 times as potent as the natural constituents of marijuana (I). However, it is only 0.01 times as potent as the 6,6-dimethyl compound IIe. It is thus seen that, although the 6,6-dinor compounds show good activity, maximum activity in II occurs when R is methyl; smaller or larger substituents result in a decrease in activity. The triol intermediate V was inactive at doses five times those at which III was active.

Experimental Section‡

2,6-Dihydroxy-4-(1,2-dimethylheptyl)-3′,4′,5′,6′-tetrahydro-2′-hydroxymethyl-5′-methylbiphenyl (V). A solution of 62.5 g of the lactone IV[4] in 220 ml of dry THF was added with cooling to a stirred suspension of 26.5 g of $LiAlH_4$ in 1 l. of THF over a period of 45 min. The mixture was heated at reflux for 2.5 hr after addition was complete. The mixture was cooled, and 100 ml of EtOAc was slowly added, followed by 75 ml of saturated Na_2SO_4 solution. The mixture was filtered, and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in 1:1 C_6H_6–Et_2O, washed with dilute HCl and H_2O, dried ($MgSO_4$), and concentrated *in vacuo* to give a dark brown oil. The oil was dissolved in a minimum of Et_2O and hexane was added until turbid. On standing the triol crystallized: 32.2 g; mp 149–152°. Recrystallization from hexane–Et_2O raised the melting point to 153–155°; the ir spectrum shows no carbonyl absorption but a very strong OH band; mass spectrum *m/e* 360 (M^+). *Anal.* ($C_{23}H_{30}O_3$) C, H.

1-Hydroxy-3-(1,2-dimethylheptyl)-7,8,9,10-tetrahydro-9-methyl-6*H*-dibenzo[*b*,*d*]pyran (III). A solution of 8.0 g of V in 200 ml of dioxane, 200 ml of H_2O, and 20 ml of dilute HCl was refluxed for 3 hr. The dioxane was evaporated *in vacuo*, the aqueous residue was extracted three times with Et_2O, and the combined extracts were washed with H_2O until neutral, dried ($MgSO_4$), and evaporated to give 6.0 g of crude product. The product was isolated by chromatography on a silica gel "dry-column"[8] using $CHCl_3$ as eluent: 2.4 g; bp 185–187° (0.025 mm); mass spectrum *m/e* 384 (M^+). *Anal.* ($C_{23}H_{34}O_2$) C, H.

Cyclization could also be carried out by heating under N_2 at 200° for several hours, by refluxing with *p*-toluenesulfonic acid in toluene, or treatment with BF_3 etherate, but these gave lower yields and more complex mixtures. Some cyclization was also observed on heating V with Ac_2O in pyridine; the resulting mixture contained some III, as shown by gc and mass spectrum (*m/e* M^+ 384).

Acknowledgments. We wish to thank Mr. E. Macko and Mr. P. J. Fowler for the biological test results.

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‡Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Boiling points and melting points are uncorrected. Elemental analyses were performed by the Analytical Department of Smith Kline & French Laboratories and where analyses are indicated by the symbols of the elements, analytical results for the elements were within ±0.4% of the theoretical values. Mass spectra were obtained on a Hitachi Perkin-Elmer RMN 6E spectrometer. Nmr spectra were obtained on a Varian T-60 instrument (Me_4Si). Ir and nmr spectra of all compounds were consistent with the assigned structures.

Studies on Anticoccidial Agents. 2. Synthesis and Anticoccidial Activity of Pyridoxol Analogs

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The previous paper[1] has described the syntheses and the anticoccidial properties of 4-deoxypyridoxol (4-DOP) and its esters. However, some of these compounds were found to be toxic as potent antagonists of vitamin B_6 in chicks. Thus it was of interest to explore the possibility of modifications at the 4 position of 4-DOP in order to obtain more desirable anticoccidial derivatives.

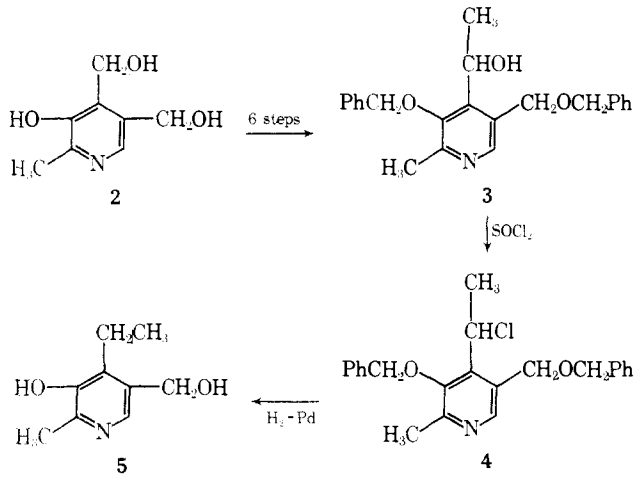
The compounds prepared herein are listed in Table I. α^4-*O*-Methylpyridoxol[2] (1, R = CH_2OMe) is known to be a vitamin B_6 antagonist in some mammals and 4-trifluoromethyl[3] (1, R = CF_3), 4-hydroxy[4] (1, R = OH), and 4-deoxymethyl[5] (1, R = H) derivatives have also been found to be very weak antagonists (Scheme I).

α^4-Methyl-4-deoxypyridoxol† (5) was prepared *via* 3,α^5-*O*-dibenzyl-α^4-methylpyridoxol (3), which had been first synthesized by Korytnyk, *et al.*,[7] the α^4-methylpyridoxol derivative 3 was treated with $SOCl_2$ to afford the chlorinated compound 4 in 82% yield, which was subsequently hydrogenated in the presence of Pd/C catalyst, giving α^4-methyl-4-deoxypyridoxol (5) in 90% yield.

4-Deoxymethylpyridoxol (12, DOMP) and 4-chloro-3-hydroxy-5-hydroxymethyl-2-methylpyridine (10) were conveniently prepared from pyridoxal (6) as shown in Scheme II.

†A brief report of this compound has appeared; see ref 6.

Scheme I

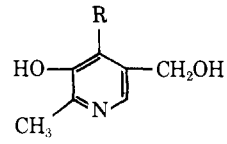


Benzoylation of 3,4-dihydroxy-5-hydroxymethyl-2-methylpyridine[4] (7) with benzoyl chloride in the presence of pyridine gave only a dibenzoate 8, which was converted to the 4-chloro derivative 9 with $POCl_3$ in 87% yield. Alkaline alcoholysis of the chloride 9 with NaOMe in MeOH under reflux gave 4-chloro-3-hydroxy-5-hydroxymethylpyridine (10), while hydrogenolysis of the chloride 9 in the presence of 5% Pd/C gave the dibenzoate 11 in 89.4% yield, which was hydrolyzed with 2 *N* HCl to produce 4-DOMP·HCl (12) in 83.8% yield.

Biological Results. The target compounds listed in Table I were tested for *Eimeria acervulina* by the procedure described in the preceding paper.[1] Compounds 1 (R = CH_2OMe, CH_2SH, CF_3), 5, and 7 showed no significant anticoccidial activity but the 4-chloro compound 10 exhibited moderate activity against *E. acervulina*. The most active compound of the group (1) was 4-DOMP·HCl (12); Replacement of the 4-methyl function in 4-DOP with H renders the compound dramatically nontoxic while still maintaining anticoccidial activity. These results suggest that the kind of the substituents in the 4 position of pyridoxol analogs accompanied by their lipid solubility seems to be important in determining their anticoccidial activity and toxicity.

The acute oral toxicity of 4-DOMP·HCl in chicks and mice was tested and LD_{50} was calculated to be 2.4 and 2.3 g/kg of body weight, respectively. The LD_{50} of 4-DOP·HCl in chicks was 1.57 g/kg; this most active compound, 4-DOMP·HCl (12), at 0.015% in feed was also effective against the quinoline derivative resistant strains of *E. acervulina* and at 0.025% in feed against *E. tenella*. Large field scale tests and floor pen trials are now being conducted in several chicken farms.

Table I. Anticoccidial Activity of Pyridoxol Analogs Modified in the 4 Position



1

No.	R	Concn of drug in feed, %	ACI
1	CH_2OMe	0.015	48
2	CH_2SH[a]	0.020	118
3	CH_2CH_3	0.015	80
4	CF_3	0.020	64
5	OH	0.020	112
6	Cl	0.015	140
7	H	0.015	195
8	CH_3	0.015	106
1-(4-Amino-2-*n*-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride		0.015	135

[a] U. Schmidt and G. Giesselmann, *Angew. Chem.*, 72, 709 (1960); G. Wendt and F. W. Bernhart, *Arch. Biochem. Biophys.*, 88, 270 (1960).

Experimental Section

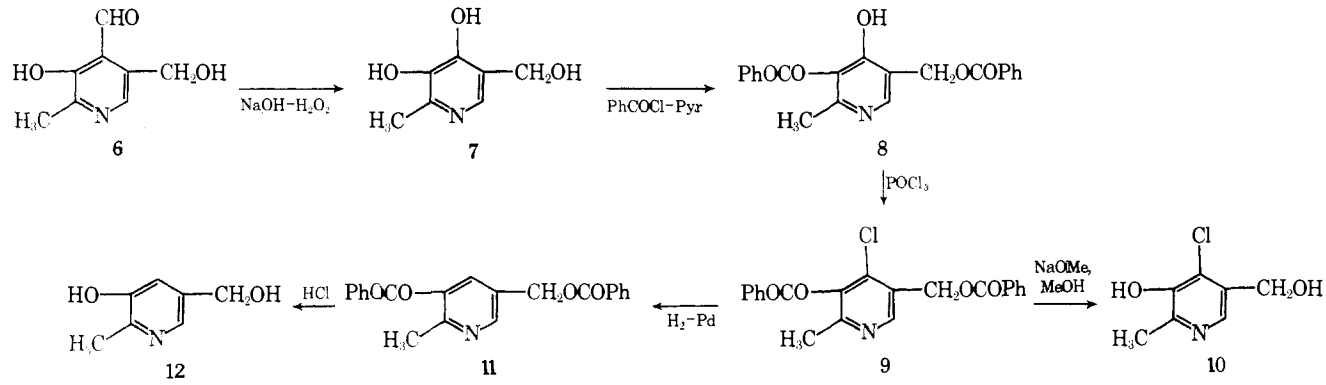
Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements are within ±0.3% of the theoretical values. All melting points are uncorrected.

3,α^5-*O*-Dibenzyl-α^4-chloro-α^4-methyl-4-deoxypyridoxol (4). To a stirred and cooled solution of 3,α^5-*O*-dibenzyl-α^4-methylpyridoxol (3, 1.1 g) in dry C_6H_6 (15 ml) was added dropwise $SOCl_2$ (1.1 ml). The mixture was stirred at room temperature for 15 min and then refluxed for 3 hr; the excess $SOCl_2$ was evaporated under reduced pressure. The residue was diluted with ice–H_2O, made alkaline with aqueous $NaHCO_3$ solution, and extracted with EtOAc. The extract was washed with H_2O, dried (Na_2SO_4), and evaporated *in vacuo* to give an oily residue, which was purified by dry silica gel chromatography (3 × 80 cm column). Elution with EtOAc–*n*-hexane (1:3) gave 0.95 g (82%) of an analytically pure product as an oil. *Anal.* ($C_{23}H_{24}NO_2Cl$) C, H, N, Cl.

α^4-Methyl-4-deoxypyridoxol (5). To a suspension of the preactivated 10% Pd/C catalyst (2.2 g) in EtOH (40 ml) containing concentrated HCl (2.2 ml) was added a solution of the chlorinated compound 4 (2.2 g) in EtOH (40 ml). The mixture was shaken in a H_2 atmosphere for 1.5 hr. After separation of the catalyst, the filtrate was evaporated into dryness to leave a colorless crystalline solid. Recrystallization from EtOH produced 1.05 g (90%) of material: mp 202–205°. *Anal.* ($C_9H_{14}NO_2Cl$) C, H, N, Cl.

3,α^5-*O*-Dibenzoyl-4-norpyridoxol (8). 4-Norpyridoxol (7, 3,4-dihydroxy-5-hydroxymethyl-2-methylpyridine) (1.55 g, 10 mmol)

Scheme II



was dissolved in pyridine (30 ml) and PhCOCl (2.8 g, 20 mmol) was added dropwise under cooling. The mixture was stirred at room temperature for 15 hr and the solvent was concentrated into a small volume *in vacuo* at 50°. The residue was diluted with H_2O and neutralized with aqueous $NaHCO_3$ solution to give a crystalline material (2.94 g, 81%), which was recrystallized from a large amount of EtOAc: mp 175°. *Anal.* ($C_{21}H_{17}NO_5$) C, H, N.

3-Benzoyloxy-5-benzoyloxymethyl-4-chloro-2-methylpyridine (9). Dibenzoate **8** (3.6 g) and $POCl_3$ (10 ml) were refluxed for 18 hr. Excess $POCl_3$ was evaporated *in vacuo*, and the residue was diluted with ice–H_2O, neutralized with aqueous dilute $NaHCO_3$ solution, and extracted with EtOAc. The extract was dried (Na_2SO_4) and the solvent was removed to give a colorless crystalline product, which was recrystallized from MeOH to produce 3.3 g (87%) of **9**: mp 126°. *Anal.* ($C_{21}H_{16}NO_4Cl$) C, H, N, Cl.

4-Chloro-3-hydroxy-5-hydroxymethyl-2-methylpyridine (10). Na (0.35 g) was dissolved in absolute MeOH (50 ml) and to this solution was added the 4-chloro compound **9** (1.9 g). The mixture was refluxed for 15 hr, the solvent was removed *in vacuo*, and the residue was diluted with H_2O (20 ml), acidified with dilute HCl, and then shaken with ether. The aqueous layer was separated, neutralized with aqueous dilute $NaHCO_3$ solution, and extracted with EtOAc. The extract was dried (Na_2SO_4) and the solvent was removed to leave an oil, which gradually crystallized. Recrystallization from EtOAc–ether gave 0.24 g (27.6%) of the product (**10**): mp 203–204°. *Anal.* ($C_7H_8NO_2Cl$) C, H, N, Cl.

3-Benzoyloxy-5-benzoyloxymethyl-2-methylpyridine (11). A solution of the 4-chloro compound **9** (3.8 g) in MeOH (500 ml) was hydrogenated in the presence of 5% Pd/C (2 g). After 30 min, the theoretical volume of H_2 was absorbed, and the solvent was removed *in vacuo*. The residue was again dissolved in EtOAc and the solution was washed with aqueous dilute $NaHCO_3$ solution and H_2O and dried (Na_2SO_4). Evaporation of the solvent left the crystalline solid, which was recrystallized from EtOAc and *n*-hexane to afford 3.1 g (89.4%) of an analytically pure product, mp 85–86°. *Anal.* ($C_{21}H_{17}NO_4$) C, H, N.

4-Deoxymethylpyridoxol (3-Hydroxy-5-hydroxymethyl-2-methylpyridine, 12). A solution of the dibenzoate **11** (1.15 g) in 2 *N* HCl (20 ml) was refluxed for 3 hr, cooled, and shaken with ether. The aqueous layer was concentrated *in vacuo* into dryness to leave a crystalline product. Recrystallization from EtOH–ether afforded 0.49 g (83.8%) of **12**: mp 167–168° (lit.[5] mp 168–170°). *Anal.* ($C_7H_{10}NO_2Cl$) C, H, N, Cl.

Acknowledgments. We wish to express our gratitude to Dr. G. Sunagawa, Director of these Laboratories, and to Dr. K. Murayama, Assistant Director, for their encouragement and discussion. We are also indebted to Miss F. Takeuchi, Messrs. T. Sakamoto, and K. Sato for their technical assistance.

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Communications to the Editor

Synthesis and Metabolic Behavior of the Suggested Active Species of Isophosphamide Having Cytostatic Activity

Sir:

Isophosphamide (**1**) is an experimentally effective antitumor agent structurally related to cyclophosphamide and is thought to exert cytotoxicity after *in vivo* metabolic transformation.[1] Some recent studies[2-4] have suggested that the activation of isophosphamide, like that of cyclophosphamide,[5-9] is caused by the enzymatic C-4 hydroxylation in animal liver. In a recent communication,[10] we have described the first chemical synthesis of the active metabolite of cyclophosphamide by a new route which may be regarded as a general method leading to 4-functionalized 1,3,2-oxazaphosphorinanes. We now apply the method to the synthesis of the suggested active species of isophosphamide and wish to report that the synthetic active species exhibited comparable antileukemic activities in both *in vivo* and *in vitro* experiments to that of cyclophosphamide and that there were significant differences not only in *in vivo* activity but also in *in vivo* metabolic behavior between isophosphamide and its active form.

O-3-Butenyl *N,N'*-bis(2-chloroethyl)phosphorodiamidate (**3**)† was prepared in 70% yield by reacting $POCl_3$ with 3-buten-1-ol and 2-chloroethylamine in CH_2Cl_2. Ozonolysis of **3** in aqueous acetone, followed by treatment with 30% H_2O_2, gave 4-hydroperoxyisophosphamide (**4**) in *ca.* 30% yield: mp 113–114° (with violent decomposition); ir_{max} (KBr) 3268, 3193, 2995, 2963, 2949, 2927, 2858, 2837, 1435, 1322, 1239, 1193, 1160, 1117, 1059, 1040, 990, 934, 879, 826, 800, 770, 744 cm^{-1}; nmr (DMSO-d_6, TMS) δ 2.09 (2 H, m, C_5-H), 2.81–4.10 (8 H, m, $2CH_2CH_2Cl$), 4.30 (2 H, m, C_6-H), 4.96 [1 H, d of t, J(P,C_4-H) = 19.0 Hz, J(C_4-H,C_5-H) = 3.0 Hz, C_4-H], 4.98 [1 H, d of t, J(P,-NH) = 19.0 Hz, J(NH,CH_2) = 5.6 Hz, NH], 11.65 (1 H, s, OOH). By the action of Fe^{2+} ($FeSO_4$) or Cu^+ (CuCl), **4** was converted into 4-ketoisophosphamide (**5**)[4] in excellent yield, while treatment of **4** with triethyl phosphite in CH_2Cl_2 at 0° resulted in the quantitative formation of 4-hydroxyisophosphamide (**2**) as fine needles: mp 74–75° dec; ir_{max} (KBr) 3313, 3285, 2990, 2950, 2931, 2888, 2860, 1444, 1313, 1262, 1234, 1215, 1195, 1109, 1063, 1044, 975, 921, 887, 810, 772, 743, 715 cm^{-1}; nmr (D_2O, DSS) δ 1.99 (2 H, m, C_5-H), 2.75–3.90 (8 H, m, $2CH_2CH_2Cl$), 4.0–4.9 (2 H, m, C_6-H), 5.05 [1 H, d of t, J(P,C_4-H) = 18.0 Hz, J(C_4-H,C_5-H) = 3.5 Hz, C_4-H]. In contrast to the behavior of 4-hydroperoxycyclophosphamide,[11] treatment with aqueous alkali (Na_2CO_3) converted **4** into a cyclic peroxide **6** in good yield: mp 127–129° dec; ir_{max} (KBr) 3200, 2975, 2928, 2920, 2867, 1467, 1428, 1300, 1255, 1215, 1162, 1136, 1080, 1062, 1026, 985, 917, 880, 861, 792 cm^{-1}; nmr (DMSO-d_6, TMS) δ 1.84 (2 H, m, C_5-H), 2.7–3.8 (7 H, m, $NHCH_2CH_2Cl$, NCH_2), 5.34 [1 H, d of t, J(P,C_4-H) =

†All the new compounds described in this communication gave correct elemental analyses (C, H, N, P, Cl).