Partial acid hydrolysis of VII afforded VIII, whose mass spectrum gave prominent ions at *m/e* 189, 379 and 796 (M^+), indicating that a tri-*O*-methyl-L-rhamnopyranosyl(1 → 4)-di-*O*-methyl-L-rhamnopyranosyl group was cleaved during hydrolysis. Since methylation of VIII afforded V, one may logically conclude that the L-rhamnopyranosyl(1 → 4)-L-rhamnopyranosyl group must be attached to C-4′ and that the L-rhamnopyranosyl group may be attached to C-2′ of the D-glucopyranosyl moiety of VII.

The NMR spectrum of VII showed a doublet at 4.4 ppm (1H, J = 7 Hz) and two broad singlets at 5.03 (1H) and 5.21 (2H) ppm. The doublet corresponds to the axial anomeric proton of D-glucose, and the singlets correspond to the equatorial anomeric protons of the three L-rhamnose units. Thus, the D-glucose unit is attached to II *via* a β-glycosidic linkage and to the three L-rhamnose residues *via* α-glycosidic bonds, and the chemical structure of this tetraglycoside is represented by VI.

The cytotoxic activities of I and VI are shown in Table II. Both saponins were quite effective against P-388, L-1210, and 9KB tissue culture systems. To demonstrate that this cytotoxic activity is not simply due to a detergent effect, glucoside and maltoside of II were prepared and evaluated in these test systems. Since both synthetic saponins were much less active, it appears that some unique structural features are required for optimal cytotoxic activity.

Extracts of *Dioscorea* have been widely used for the treatment of a variety of tumors in China, India, South America, and Southeast Asia (17). After completion of these studies, it was noted that I and VI had been isolated from the dried rhizomes of *Paris polyphylla* SM. obtained in a market of Katmandu, Nepal (18).

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NOTES

Phosphodiesterase Inhibition by Succinic and Related Acid Biphenylalkyl Monoesters

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Abstract □ Biphenylalkyl monoesters of succinic and related acids represent a potent new class of cyclic AMP phosphodiesterase inhibitors. The biphenyl group is necessary for potent inhibition. The spatial relationship of the carboxyl and ester groups and alkyl chain length are important to inhibitory potency.

Keyphrases □ Succinic acid biphenylalkyl monoesters—phosphodiesterase inhibition, synthesis, structure–activity relationships □ Cyclic AMP phosphodiesterase—inhibitors, succinic acid biphenylalkyl monoesters, synthesis, structure–activity relationships □ Phosphodiesterase inhibitors—succinic acid biphenylalkyl monoesters, synthesis, structure–activity relationships

Tissue cyclic AMP levels appear to be regulated by two enzymes, adenylate cyclase and phosphodiesterase, and by cellular extrusion. Phosphodiesterase catalyzes the hydrolysis of cyclic AMP to 5′-AMP. Because phosphodiesterase inhibitors can increase tissue cyclic AMP levels, these compounds are of interest as biochemical tools and as potential therapeutic agents (1–3).

Most potent cyclic AMP phosphodiesterase inhibitors are nitrogen-containing heterocycles, *i.e.*, theophylline, 3-isobutyl-1-methylxanthine, papaverine, and 4-(3-butoxy-4-methoxyphenyl)-2-imidazolidinone[1], and most contain either a 6,5- or 6,6-fused heterocyclic ring (4–6). Only a few reports described the effects of organic acids on phosphodiesterase. Citrate at high concentrations (12 m*M*) inhibited the enzyme (7). Ethacrynic acid was reported to be one-half as potent as theophylline against the enzyme from beef heart (8). Recently, acidic anti-inflammatory agents were reported to inhibit phosphodiesterase (9–11). Enzyme activation by the lipoidal organic acid, stearic acid, was observed (12), as was inhibition by unsaturated fatty acids (13).

1-(4-Biphenylyl)pentyl hydrogen succinate is a hypo-

[1] Ro 20-1724.

0022-3549/79/0700-0903$01.00/0

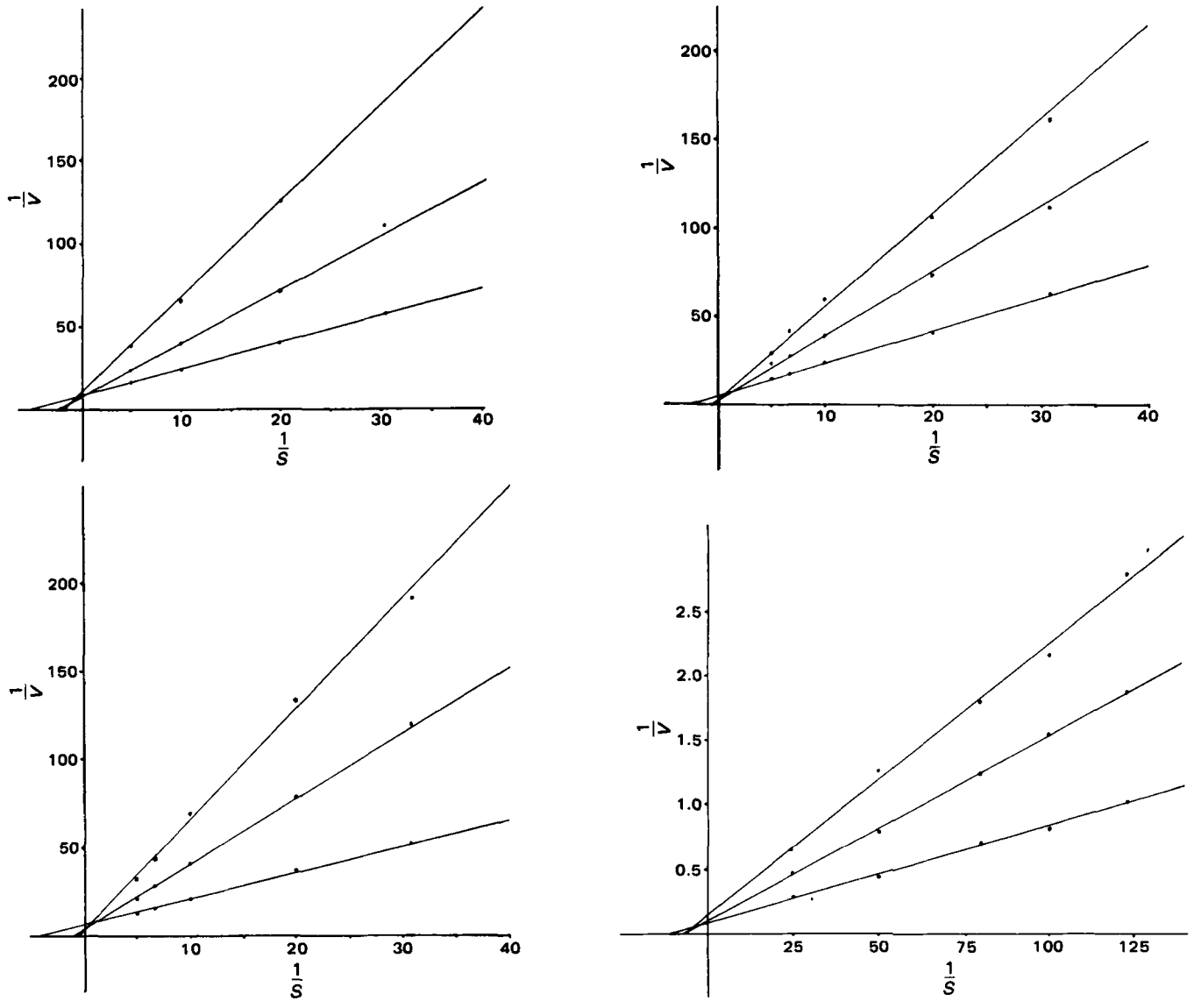
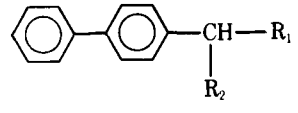
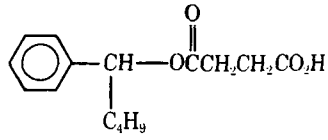


Figure 1—*Double reciprocal plots (micromoles^{-1} minute milligram of protein* versus *molarity^{-1} × 10^{-3}) at high substrate concentrations for 150 and 300 μM Ib (upper left), 70 and 110 μM Ie (upper right), and 350 and 500 μM Id (lower left) and at a low substrate concentration for 50 and 100 μM Ib (lower right).*



Ia: R_1 = —OC(=O)$CH_2CH_2CO_2H$, R_2 = methyl
Ib: R_1 = —OC(=O)$CH_2CH_2CO_2H$, R_2 = *n*-butyl
Ic: R_1 = —OC(=O)$CH_2CH_2CO_2H$, R_2 = *n*-hexyl
Id: R_1 = —OC(=O)CH=$CHCO_2H$ (*cis*), R_2 = *n*-butyl
Ie: R_1 = —OC(=O)CH=$CHCO_2H$ (*trans*), R_2 = *n*-butyl
If: R_1 = —OC(=O)CH=$CHCO_2H$ (*trans*), R_2 = *n*-hexyl



II

cholesterolemic agent previously reported to inhibit β-hydroxy-β-methylglutaryl coenzyme A reductase (14). The purpose of this investigation was to examine the phosphodiesterase inhibition by *Ib* and related acids.

RESULTS AND DISCUSSION

Compound *Ib* was a potent beef heart phosphodiesterase[2] inhibitor (Table I). Closely related *Ia*, *Ic*–*Ie*, and II were also examined for effects on the enzyme[3]. Lineweaver–Burke plots were obtained for *Ib*, *Id*, and *Ie* (Fig. 1). The I_{50} values were determined at substrate concentrations of 100 and 1 μ*M* for *Ia*–*Ie*. Most phosphodiesterase preparations, including bovine heart phosphodiesterase, contain both high and low affinity enzymes or enzyme forms (5, 6). The preparations could be assayed at a low cyclic AMP concentration to observe effects on the high affinity form and at high substrate concentrations (actually, this high concentration reflected total enzyme activity) to observe effects on the low affinity enzyme. Additionally, miscellaneous acids were examined for effects on phosphodiesterase to substantiate *Ib*–*Ie* specificity. Finally, structure–activity relationship analysis of *Ia*–*Ie* led to the synthesis of *If*, the most potent inhibitor yet uncovered in this series.

The discovery that *Ib* inhibited phosphodiesterase raised the following question: Would other organic acids, especially arylalkyl organic acids, in view of the known acidic anti-inflammatory agent effect, inhibit the enzymes? The failure of all eight diverse acids to inhibit phosphodiesterase indicated that this is not a general organic acid effect (Table I).

[2] Sigma Chemical Co.

[3] These results did not correlate with the potency of these compounds as inhibitors of β-hydroxy-β-methylglutaryl coenzyme A reductase (14, 15).

Table I—Phosphodiesterase Inhibition [a]

Compound [b]	I_{50}, μM	
	1 μM Cyclic AMP [c]	100 μM Cyclic AMP [c]
Ia	514 ± 56 [d]	1215 ± 103
Ib	64 ± 2	205 ± 6
Ic	49 ± 2	95 ± 7
Id	121 ± 15	402 ± 27
Ie	29 ± 2	93 ± 10
If	16 ± 2	25 ± 3
II	823 ± 68	>1000 [e]
Theophylline	306 ± 24	917 ± 25
3-Isobutyl-1-methyl-xanthine	10 ± 1	52 ± 1

[a] Bovine heart phosphodiesterase was used. [b] The following acids at 5×10^{-4} M, using 100 μM cyclic AMP, showed less than 5% enzyme inhibition: *p*-chlorophenoxyacetic, cinnamic, *p*-hydroxyphenylacetic, monomethyl adipate, α-naphthylacetic, α-phenylbutyric, phenylpropionic, and 2-pyridylacetic. [c] Substrate concentration. [d] Data are expressed as the mean ± *SE* of three determinations. [e] Sixteen-percent inhibition at 1000 μM; a solubility problem was encountered at higher concentrations.

Additional specificity evidence was indicated by the lack of succinate analog activity where biphenyl was replaced by phenyl (II) and where the *n*-butyl chain was replaced by methyl (Ia, Table I). Furthermore, if the inhibition was *via* an enzyme–inhibitor complex, there should be a variation in potency among stereoisomers. In fact, the fumarate (*trans*-isomer) analog Ie was four times as potent as the maleate (*cis*-isomer) analog Id, with the succinate Ib intermediate between the two.

Attempts to establish the inhibition type *via* Lineweaver–Burke plots were not definitive (Fig. 1). Beef heart phosphodiesterase inhibition by Ib appeared to be competitive or partially competitive at both low and high substrate concentrations. Replotting the data using Hofstee plots did not improve identification of the inhibition type. The inability to obtain definitive curves may have been due to assay error, enzyme preparation crudeness, or the presence of additional enzyme forms.

The most striking structure–activity relationships for this series (Table I) can be summarized as follows: the biphenyl moiety is necessary (Ib *versus* II), a *trans*-configuration of the carboxyl and ester groups is optimal (Ie > Ib > Id), and an increased alkyl chain length is advantageous (Ic > Ib > Ia). These results suggested the synthesis of If[4].

Compound If is almost equipotent with the very potent, standard phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine against the high affinity enzyme and is more potent against the low affinity enzyme. The ability of these compounds to inhibit cyclic AMP phosphodiesterase should be considered when interpreting pharmacological studies.

EXPERIMENTAL

Chemistry[5]—Compounds Ia–Ic and II were prepared as previously described (15). Compounds Id and Ie were synthesized as previously described (14).

Compound If, mp 82–84°, was prepared using the method described for Ie (14); NMR: δ 0.60–2.20 [broad, 13H, $CH_3(CH_2)_5$], 5.90 (t, J = 6 Hz, 1H, HCO_2C–), 6.95 [s, 2H, —CH=CH—], 7.20–7.80 [m, 9H, aromatic], and 8.45 [s, 1H, CO_2H] ppm.

Anal.—Calc. for $C_{23}H_{26}O_4$: C, 75.4; H, 7.1. Found: C, 75.1; H, 7.4.

Enzyme Studies—The assay (16) was used as previously described (17) employing bovine heart phosphodiesterase[2].

[4] Further chain elongation would likely reduce solubility beyond workable limits.

[5] Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Atlanta, Ga. NMR spectra were determined using a Perkin-Elmer R-24 spectrometer in deuterochloroform with tetramethylsilane as the internal reference.

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