produced in man by administration of *dl*-methadone has yet to be assessed.

A relatively minor pathway involved the oxidation of methadone to 4-dimethylamino-2,2-diphenylvaleric acid (**8**). This acidic metabolite appeared to be subsequently N-demethylated, in part, to an intermediate metabolite (not isolated), 4-methylamino-2,2-diphenylvaleric acid, which by ring closure yielded metabolite **4**, 1,5-dimethyl-3,3-diphenyl-2-pyrrolidone. The conversion of methadone to a carboxylic acid has some precedent in the known enzymatic oxidation of acylbenzenes to benzoic acid.[18]

In the studies reported here, no methadone *N*-oxide was found in freshly obtained or frozen samples. Upon standing at room temperature, however, the urine samples appeared to develop a component which had characteristics similar to methadone *N*-oxide. These results are at variance with those of Beckett, Vaughan, and Essien[12] who report methadone *N*-oxide to be an important metabolite of methadone.

Despite the relatively large number of methadone metabolites found in human urine, they probably do not account for the entire daily maintenance dose. Further studies, probably involving labeled drug, will be required to answer this question.

Acknowledgment. My interest in methadone research began 20 years ago in the laboratory of Dr. Albert Pohland, to whom I express my appreciation for his continued interest and support. I also wish to acknowledge the advise and encouragement I have received from Dr. Robert E. McMahon and Dr. Irwin H. Slater. The authors are indebted to Dr. David Blake, Man Alive Research, Inc., and University of Maryland School of Pharmacy for selecting the subjects and for supervision of the urine collection and shipment.

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Structural Requirements for Tumor-Inhibitory Activity among Benzylisoquinoline Alkaloids and Related Synthetic Compounds†

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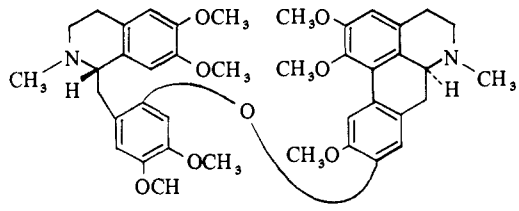
The results of a study directed toward determination of the structural requirements for tumor-inhibitory activity among benzylisoquinoline alkaloids and related synthetic compounds are reported. Monomeric benzylisoquinolines and aporphines appear to have no inhibitory activity against the W-256 tumor system. In contrast, significant inhibitory activity is manifested by a wide range of bis(benzylisoquinoline) derivatives. The comparable activity of *dl*-*O*-methyldauricine (**11a**) and of **14** with those of tetrandrine (**2a**) and thalidasine (**6**) indicates that a macrocyclic ring is not required. Methylation of the nitrogen atoms does not appear to be necessary, for **9**, **11b**, and **12** each show inhibitory activity. The significant activity of **9** and **12** indicates the apparent absence of stereospecificity requirements for biological activity in the series. One possible rationalization, that bis(benzylisoquinolines) may exert their tumor-inhibitory activity by a mechanism involving initial metabolic dehydrogenation to bis(dihydroisoquinolinium) derivatives, is rendered unlikely by the observed inactivity of **10** and the significant activity of **11c**.

Interest in the bis(benzylisoquinoline) alkaloids was stimulated by our report in 1966 that the alkaloids thalicarpine[2] and *dl*-tetrandrine[3] have significant inhibitory activity against the Walker intramuscular carcinosarcoma 256 in the rat over a wide dosage range.[4,5] Subsequent studies revealed that the dextrorotatory enantiomer, tetrandrine, shows activity of the same order as the racemate. Thalicarpine (**1**)[6] and tetrandrine (**2a**)[7] have undergone extensive preclinical toxicological studies and have been selected for clinical evaluation now in progress under the auspices of the National Cancer Institute.

In view of the increasing significance of the biological properties of the benzylisoquinoline alkaloids, we deemed it important that studies be undertaken which are directed toward elucidation of the mode of action of the active alkaloids. We report herein some recent findings concerning the first step, *viz.*, the definition of structural and stereochemical requirements for tumor-inhibitory activity.

The monomeric benzylisoquinolines and aporphines appear to have no tumor-inhibitory activity against the W-256 tumor system. For example, *dl*-1-benzyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**3**),[8] in the form of its pamoate [*i.e.*, salt of 4,4′-methylenebis(3-hydroxy-2-naphthoic acid)], was found to be toxic at doses equivalent to 50 mg/kg of free base and higher and inactive at lower doses. None of the additional dozen or more benzyliso-

† Tumor Inhibitors. 89. For paper 88, see ref 1.



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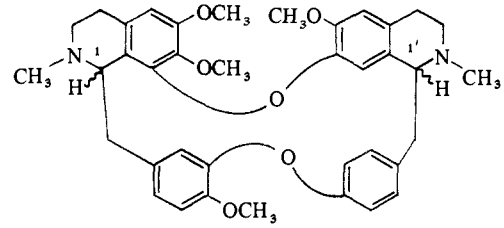
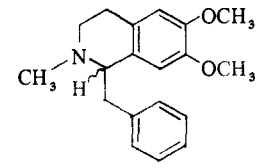


2a, 1-*S*, 1'-*S*
b, 1-*R*, 1'-*R*



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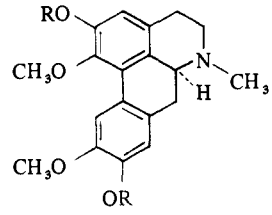
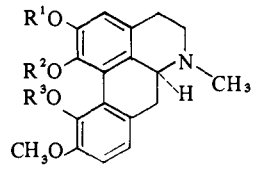
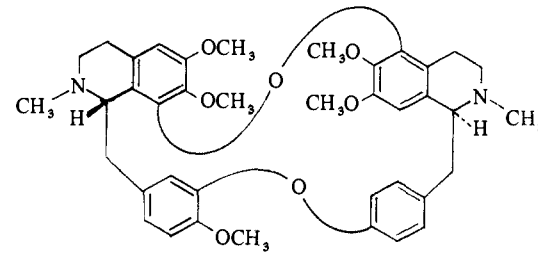


4a, R = CH_3
b, R = H



5a, $R^1 = R^3 = CH_3$; R^2 = H
b, $R^1 = R^2 = CH_3$; R^3 = H
c, R^1, R^2 = $-CH_2-$; R^3 = H



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quinoline derivatives tested nor the aporphine alkaloids glaucine (**4a**), boldine (**4b**), corydine (**5a**), isocorydine (**5b**), or bulbocapnine (**5c**) have shown W-256 inhibitory activity in the NCI program.

The comparable activities of **1** and **2a** established earlier that the component moieties of the dimeric alkaloids could be either benzyltetrahydroisoquinoline or aporphine. Furthermore, the inhibitory activity of thalidasine (**6**)[9] indicated that the size of the macrocyclic ring in bis(benzylisoquinoline) alkaloids containing two diaryl ether linkages was not a crucial factor. To elaborate further the structural requirements for tumor-inhibitory activity among bis(benzylisoquinoline) derivatives, the total synthesis of *dl-O*-methyldauricine (**11a**, *RR,SS*) and several structural variants was undertaken.

l-O-Methyldauricine has been synthesized earlier by the Ullmann-type condensation of derivatives of alkaloids obtained from natural sources.[10,11] Drawbacks of this approach are the inaccessibility of the starting materials and the low yield of the final step, the formation of the diaryl ether link. *O*-Methyldauricine and dauricine have also been generated by total synthesis as constituents of mixtures of racemates,[12-16] but no separation of the racemate mixtures has been reported.

Our syntheses were achieved by sequences beginning with simple diphenyl ethers followed by the simultaneous elaboration of the isoquinoline moieties. In this manner, and after suitable separations, the synthesis and isolation of *dl-O*-methyldauricine (**11a**, *RR,SS*) and of one racemate of **11b** and of **14** were accomplished in the multigram scale needed for *in vivo* biological testing.

The Ullmann condensation of guaiacol with bromobenzene gave 2-methoxy diphenyl ether.[17] Acetylation with acetyl chloride-aluminum chloride gave 2-methoxy-5,4'-diacetyl diphenyl ether.[18] The diketone was oxidized to 2-methoxy-5,4'-dicarboxymethyl diphenyl ether (**7a**) by Willgerodt oxidation with sulfur-morpholine[18] and the corresponding diacyl chloride **7b** was prepared with thionyl chloride-pyridine. The diacyl chloride was treated *in situ* with 2 equiv each of β-(3,4-dimethoxyphenyl)ethylamine and triethylamine in chloroform to give diamide **8**.[12,19] Bischler-Napieralski cyclodehydration with phosphorous oxychloride in refluxing benzene (N_2) gave bis(benzyldihydroisoquinoline) **9**,[12] isolated as the dioxalate. Dissolution of **9** in an excess of iodomethane resulted in quantitative separation of dimethiodide **10**.

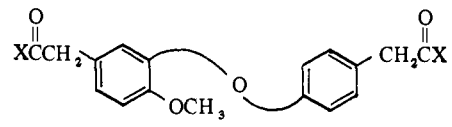
Reduction of **10** with sodium borohydride-methanol and plc on alumina plates gave a mixture of **11a** diastereomers.[16] Separation of one racemate of **11a** was achieved by recrystallization of the dioxalate mixture from acetone-methanol. The nmr spectrum of the liberated base was identical with that of *l-O*-methyldauricine.[11] The separated racemate was thus *dl-O*-methyldauricine, a racemate which had previously eluded isolation in a homogeneous form.[12,13]

Treatment of **10** with an excess of methylmagnesium iodide in ether (N_2) and plc on alumina plates gave a mixture of **11c** diastereomers. Attempts to separate the mixture *via* the oxalates, citrates, or succinates were unsuccessful. The diastereomeric mixture of **11c** was converted to its crystalline pamoate.

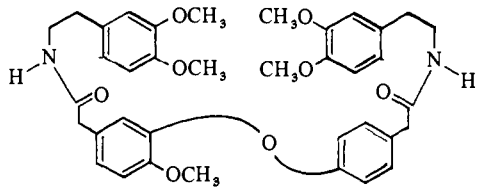
Reduction of **9** with sodium borohydride-methanol and plc on silica gel plates gave a mixture of **11b** diastereomers.[12] Separation of one racemate of **11b** dioxalate was accomplished by recrystallization of the dioxalates. The liberated base was converted to *dl-O*-methyldauricine by treatment with formalin-sodium borohydride in methanol. The product was identified by its nmr spectrum and the mixture melting point of its dioxalate with that of authentic *dl-O*-methyldauricine. Consequently, the configuration of this **11b** racemate was established as *RR,SS*.

Bis(benzylisoquinoline) **12** was prepared similarly to **9** starting from diphenyl ether and proceeding *via* 4,4'-diacetyl diphenyl ether,[20] 4,4'-dicarboxymethyl diphenyl ether,[21] and 2,2'-(oxydi-*p*-phenylene)bis[*N*-(3,4-dimethoxyphenethyl)]acetamide.[22] Cyclodehydration of this diamide to **12**[22] was achieved with phosphorous oxychloride in refluxing benzene (N_2). Treatment of **12** with excess iodomethane led to quantitative separation of dimethiodide **13**. Reduction of **13** with sodium borohydride-methanol and plc on alumina plates gave a mixture of diastereomers of **14**. Separation of one racemate of **14** dioxalate[23] from the mixture (monitored by nmr spectroscopy, the C-8 proton resonance[24] being different for each racemate) was accomplished by repeated recrystallization. The liberated base from this single dioxalate racemate exhibited C-8 proton resonance at δ 6.02.

The bis(benzylisoquinolines), characterized by satisfactory 100-MHz nmr, uv, and mass spectra, were liberated from



7a, X = OH
b, X = Cl



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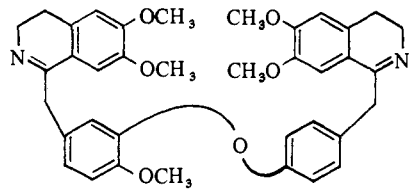

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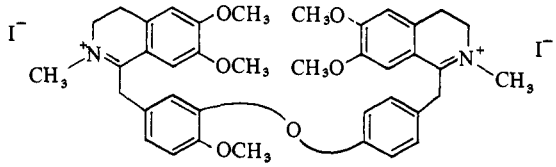

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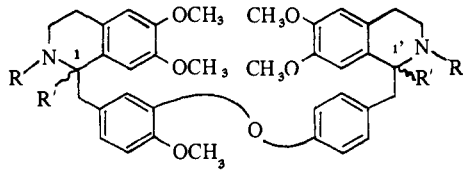
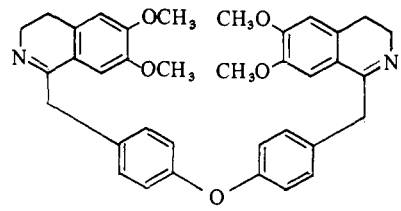


11a, R = CH_3; R′ = H
b, R = H; R′ = H
c, R = CH_3; R′ = CH_3



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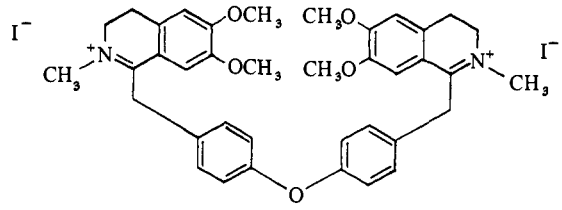

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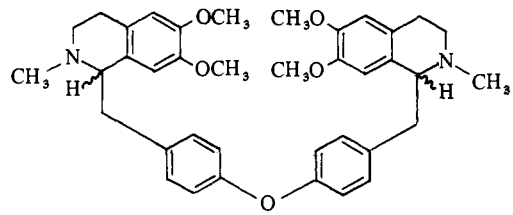

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Table I. W-256 Inhibitory Bis(benzylisoquinolines)

Compd	Dose[a]	Survivors	Animal wt differences, g ($T - C$)	Tumor evaluation, T/C	$T/C \times 100$
9	400	2/4			Toxic
	200	4/4	−12	1.9/6.9	27
	100	4/4	−7	3.3/6.9	47
11a[b]	200	0/4			Toxic
	100	3/4	−8	1.5/4.1	36
11b[b]	100	1/4			Toxic
	50	4/4	+1	1.7/4.1	41
	25	4/4	−2	2.3/4.1	56
11c[c]	400	2/4			Toxic
	200	3/4	−10	1.1/4.1	26
	100	4/4	−7	1.8/4.1	43
12	400	0/4			Toxic
	200	3/4	−25	0.2/4.1	4
	100	4/4	−15	1.7/4.1	41
14[d]	200	2/4			Toxic
	100	4/4	−15	1.3/4.1	31

[a] Dose in mg/kg of base equivalent to pamoate salt injected. [b] *RR,SS*. [c] Mixture of racemates. [d] One racemate.

the dioxalates with NH_4OH–$CHCl_3$ and were converted to their pamoates for biological testing. The assay‡ results for the active compounds are shown in Table I.

The testing results indicate that significant tumor-inhibitory activity is manifested by a surprisingly wide range of bis(benzylisoquinoline) derivatives. The inhibitory activity of **11a** and **14** indicates that a macrocyclic ring is not required; the bis(benzylisoquinolines) containing one diaryl ether linkage are quite active. Methylation of the nitrogen atoms does not appear to be necessary, for **9**, **11b**, and **12** each show significant activity. Furthermore, the activity of **9** and **12** indicates the apparent absence of stereospecificity requirements for biological activity in this series, as suggested earlier by the finding that pheanthine (**2b**) shows appreciable activity.[5]

The foregoing results and the fact that the W-256 tumor system is known to be sensitive to alkylating agents led us to entertain the hypothesis that bis(benzylisoquinoline) alkaloids may exert their tumor-inhibitory activity by a stepwise sequence leading to bisalkylation of biological macromolecules involved in growth regulation.[26,27] One possibility would involve prior metabolic dehydrogenation to the bis(dihydroisoquinolinium) system present in **10**; such a system would be expected to show great reactivity toward attack by nucleophiles at C-1 and C-1′.[28] In the event, **10** proved to be inactive and nontoxic in doses up to 560 mg/kg. Furthermore, **11c**, which would be unlikely to undergo ready metabolic transformation to a compound like **10**, was found to show a high order of W-256 inhibitory activity. Investigations are under way to evaluate other hypothetical mechanisms for the tumor-inhibitory activity of bis(benzylisoquinoline) alkaloids.

Experimental Section

Melting points were determined on a calibrated Thomas-Hoover (Uni-Melt) apparatus. Uv spectra were measured on a Beckman DK-2A recording spectrophotometer. Mass spectra were determined on a Hitachi Perkin-Elmer RMU-6E spectrometer. Nmr spectra were determined on a Varian Associates HA-100 spectrometer. Plc was performed on 1.5-mm alumina (Type T) F-254 20 × 20 cm (EM Re-

‡ Assays were performed under the auspices of Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, by the procedures described in ref 25. A compound is considered to show significant *in vivo* activity against the W-256 system if it causes reduction of tumor weight in treated (T) animals to 42% or less of the tumor weight in control (C) animals (*i.e.*, $T/C \leq 42\%$).

agents) plates or on 2.0-mm silica gel F-254 20 × 20 cm (EM Reagents) plates. Tlc was conducted on alumina (Type T) F-254 5 × 20 cm (E. Merck AG, Darmstadt) plates. Visualization of the compounds on the plates was accomplished with a Chromato-Vue (Ultra-Violet Products, Inc., San Gabriel, Calif.) viewbox with 254-nm light. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., and satisfactory results for C, H, and N were obtained. All pamoates were crystallized from MeOH unless otherwise stated.

Pamoate of *dl*-1-Benzyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (3). To benzylisoquinoline **3**[8] (2.00 g) in MeOH (25 ml) and glacial HOAc (0.84 g) was added a solution of disodium pamoate (2.90 g) in MeOH (40 ml). The separated salt (2.82 g, 85%, MeOH) had mp 168–170°. *Anal.* ($C_{19}H_{23}NO_2 \cdot 0.5C_{23}H_{16}O_6$).

2-Methoxy diphenyl ether was prepared with copper(I) oxide in collidine.[17] The yield was 58%; mp 76–78° (lit.[17] mp 78°).

2-Methoxy-5,4′-diacetyl diphenyl ether was synthesized from 2-methoxy diphenyl ether by the literature procedure[18] except that the solvent was 1,2-dichloroethane at room temperature. The yield was 91% (95% EtOH); mp 142–143° (lit.[18] mp 144°).

2-Methoxy-5,4′-dicarboxymethyl diphenyl ether (7a) was obtained from 2-methoxy-5,4′-diacetyl diphenyl ether in 79% (HOAc) yield, mp 174–176° (lit.[18] mp 173–175°).

Diamide 8. Diacid 7a was converted to the diacyl chloride 7b and the latter was converted to **8**. The yield was 78%, mp 137–139° (lit.[19] mp 139–140°).

Bis(benzyl-3,4-dihydroisoquinoline) 9 was obtained by refluxing for 3 hr (N_2) a stirred suspension of 8 (1.50 g) with $POCl_3$ (8.00 g) in dry benzene (40 ml). The product was isolated as its dioxalate (1.48 g, 80%, acetone–MeOH), mp 133–135° dec. *Anal.* ($C_{37}H_{38}N_2O_6 \cdot 2C_2H_2O_4 \cdot 1.5H_2O$). The pamoate (EtOH) had mp 219–221° dec. *Anal.* ($C_{37}H_{38}N_2O_6 \cdot C_{23}H_{16}O_6 \cdot H_2O$). The liberated base from dioxalote 9 gave uv (MeOH) 276 nm (log ε 4.17), 309 (4.04); mass spectrum *m/e* 606 M^+.

Dimethiodide 10. Treatment of 9 (prepared from 6.00 g of **8**) with methyl iodide (30 ml) afforded a yellow suspension. The mixture was stirred at room temperature (N_2) for 5 hr. The yield of **10** (8.47 g) was essentially quantitative: uv (MeOH) 245 nm (log ε 4.41), 309 (4.19), 363 (4.11); mp 165–170°. *Anal.* ($C_{39}H_{44}I_2N_2O_6 \cdot H_2O$) C, H, N, I.

dl-O-Methyldauricine 11a. A stirred suspension of dimethiodide **10** (10.00 g) in MeOH (300 ml) was kept at room temperature until most of **10** had dissolved. The stirred solution was cooled to ice-bath temperature and distilled H_2O (20 drops) was added. After portionwise addition of $NaBH_4$ (10.00 g), the solution was stirred at room temperature for 1 hr. Work-up and plc on alumina plates (1% MeOH–$CHCl_3$) gave a mixture of **11a** racemates (5.12 g). Two crystallizations on the dioxalates (acetone–MeOH) gave one 11a racemate dioxalate (1.66 g, 50%), mp 210–212°. *Anal.* ($C_{39}H_{46}N_2O_6 \cdot 2C_2H_2O_4 \cdot 0.5H_2O$). The pamoate had mp 198–200°. *Anal.* ($C_{39}H_{46}N_2O_6 \cdot C_{23}H_{16}O_6 \cdot 2H_2O$). The liberated base from this racemate dioxalate showed an identical nmr spectrum[11] and tlc retention time with that of authentic *l-O*-methyldauricine: uv (MeOH)[29] 284 nm (log ε 4.04); mass spectrum[29] *m/e* (rel intensity) 637 M^+ (<1%), 206 (100%).

l-O-Methyldauricine. A solution of dauricine (0.018 g) in anhydrous MeOH (10 ml) was treated with an excess of ethereal diazomethane. The solution was kept at 0° for 120 hr, after which time the reaction appeared to have proceeded to completion (tlc). The product was purified on alumina plates (1% MeOH–$CHCl_3$) to give *l-O*-methyldauricine (0.011 g) with physical constants cited above.

Bis(benzyltetrahydroisoquinoline) 11c. Dry tetrahydrofuran (240 ml) and then **10** (10.00 g) were added portionwise to ethereal methylmagnesium iodide prepared from Mg turnings (5.50 g) and methyl iodide (15 ml) in anhydrous diethyl ether (150 ml). The stirred gray suspension was kept (N_2) at room temperature for 2 hr. The excess Grignard reagent was decomposed by careful addition of distilled H_2O to the stirred mixture at ice-bath temperature. The organic solvents were removed *in vacuo* and work-up followed by purification of the product on alumina plates (1% MeOH–$CHCl_3$) gave a mixture of 11c racemates (6.58 g). The pamoate (4.74 g, 46%, mp 195–197°) was obtained from the 11c mixture. *Anal.* ($C_{41}H_{50}N_2O_6 \cdot C_{23}H_{16}O_6 \cdot H_2O$). The liberated base from pamoate of 11c gave uv (MeOH) 283 nm (log ε 3.95); nmr δ 1.34, 1.40 (6 H, 2 CCH_3), 2.40, 2.47 (6 H, 2 NCH_3), 3.66–3.76 (15 H, 5 OCH_3); mass spectrum *m/e* (rel intensity) 666 M^+ (<1%), 220 (100%).

Bis(benzyltetrahydroisoquinoline) 11b. Distilled H_2O (50 drops) and then $NaBH_4$ (7.00 g) were added slowly to a stirred MeOH solution (500 ml) of 9 (prepared from 10.00 g of **8**) at ice-bath temperature. The stirred colorless solution was kept at room temperature for *ca.* 1 hr. Work-up followed by plc on silica gel (30% MeOH–$CHCl_3$) plates gave a mixture of **11b** racemates (6.88 g). Two crystallizations of the dioxalates (MeOH) gave one **11b** racemate dioxalate (3.02 g, 68%), mp 189–191°. *Anal.* ($C_{37}H_{42}N_2O_6 \cdot 2C_2H_2O_4 \cdot H_2O$). The pamoate had mp 205–207°. *Anal.* ($C_{37}H_{42}N_2O_6 \cdot C_{23}H_{16}O_6 \cdot 4.5H_2O$). The liberated base from dioxalate racemate 11b gave uv (MeOH) 283 nm (log ε 3.96); nmr δ 3.71, 3.75, 3.77 (3 H, 6 H, 6 H, 5 OCH_3); mass spectrum *m/e* (rel intensity) 610 M^+ (<1%), 192 (100%). A MeOH solution (4 ml) of this 11b racemate (0.09 g) was cooled to ice-bath temperature and 37% formalin (1.00 g) was added. This stirred solution was kept at ice-bath temperature for 10 min and $NaBH_4$ (0.57 g) was then added over a 1-hr period. Work-up and plc purification on silica gel (10% MeOH–$CHCl_3$) gave 0.03 g of product with nmr and mass spectra identical with that of *dl-O*-methyldauricine (*vide supra*). The melting point (209–211°) of the dioxalate was undepressed by admixture with the dioxalate of authentic *dl-O*-methyldauricine.

4,4′-Diacetyl diphenyl ether was prepared from diphenyl ether in 93% (95% EtOH) yield, mp 100–102° (lit.[21] mp 101–102°).

4,4′-Dicarboxymethyl diphenyl ether was synthesized from 4,4′-diacetyl diphenyl ether in 62% (HOAc) yield, mp 230–231° (lit.[21] mp 227.5–230°).

2,2′-(Oxydi-*p*-phenylene)bis[*N*-(3,4-dimethoxyphenethyl)]-acetamide was prepared from the diacyl chloride of 4,4′-dicarboxymethyl diphenyl ether. The crude diamide (10.00 g) was purified by column chromatography on silica gel [200 g, 0.05–0.20 mm (70–325 mesh ASTM) E. Merck AG, Darmstadt] by elution with 5% MeOH–$CHCl_3$ (1000 ml). The eluted material was decolorized with Darco G-60 charcoal in boiling ethyl acetate and then recrystallized twice (ethyl acetate). The yield was 6.67 g (67%); mp 97–99° (lit.[22] mp 99°).

Bis(benzyl-3,4-dihydroisoquinoline) 12 was obtained from 2,2′-(oxydi-*p*-phenylene)bis[*N*-(3,4-dimethoxyphenethyl)]acetamide (1.50 g). The dioxalate was isolated in 55% yield (MeOH), mp 216–217° dec. *Anal.* ($C_{36}H_{36}N_2O_5 \cdot 2C_2H_2O_4$). The pamoate had mp 192–195° dec. *Anal.* ($C_{36}H_{36}N_2O_5 \cdot C_{23}H_{16}O_6 \cdot 2H_2O$). The liberated base from dioxalate **12** gave uv (MeOH) 275 nm (log ε 4.12), 308 (4.01); mass spectrum *m/e* 576 M^+.

Dimethiodide 13 was synthesized by treating **12** [prepared from the precursor diamide (10.00 g)] with iodomethane (60 ml) and stirring the yellow suspension for 5 hr at room temperature (N_2). The yield (13.95 g) was essentially quantitative: mp 170–172°; uv ($CHCl_3$) 243 nm (log ε 4.65), 304 (4.32), 365 (4.26). *Anal.* ($C_{38}H_{42}I_2N_2O_5 \cdot 2H_2O$). The monobenzene solvate (benzene–MeOH) had mp 176–178°. *Anal.* ($C_{38}H_{42}I_2N_2O_5 \cdot C_6H_6$) C, H, N, I.

Bis(tetrahydroisoquinoline) 14. Dimethiodide 13 (13.70 g) was converted to **14** with $NaBH_4$ (13.70 g) in MeOH (430 ml). Plc of **14** on alumina (1% MeOH–$CHCl_3$) plates gave a mixture of 14 racemates (7.04 g) and several recrystallizations of the dioxalates (acetone–MeOH) gave **14** dioxalate (1.64 g, 36%, mp 193–195°), indicated by nmr spectroscopy to be essentially a single racemate. *Anal.* ($C_{38}H_{44}N_2O_5 \cdot 2C_2H_2O_4 \cdot 1.5H_2O$). The pamoate had mp 198–200°. *Anal.* ($C_{38}H_{44}N_2O_5 \cdot C_{23}H_{16}O_6 \cdot 1.5H_2O$). The liberated base from dioxalate racemate **14** gave uv (MeOH) 284 nm (log ε 3.91); nmr δ 2.49 (6 H, 2 NCH_3), 3.57, 3.78 (6 H, 6 H, 4 OCH_3), 6.02 (s, 2 H), 6.48 (s, 2 H); mass spectrum *m/e* (rel intensity) 607 M^+ (<1%), 206 (100%). Several additional recrystallizations of a small sample of the dioxalate (acetone–MeOH) gave a product which sintered at 134–137° and showed mp 201–202° (lit.[23] 205°).

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Structure–Activity Relationship of Chloramphenicols†

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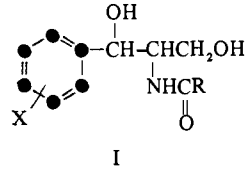
To obtain greater insight into the structure–activity relationship of chloramphenicol, 37 derivatives (including the parent drug) have been tested *via* the microbial kinetic technique against *Escherichia coli*. Two quantitative structure–activity relationships have been formulated: one for the side chain modified derivatives and one for the derivatives modified in the 4 position of the ring. From these results it is apparent that inductive effects of the acyl group in the side chain are important and that hydrophobic properties of the side chain are less important. For substituents in the 4 position, hydrophobic properties are most important. The first reported instance of a derivative more active than the parent drug is given for the compound having $NHCOCF_3$ instead of $NHCOCHCl_2$ of the natural drug. The trifluoro derivative is 1.7 times more active against *E. coli* than chloramphenicol.

Since the discovery[1-3] and synthesis of chloramphenicol by workers at the Parke Davis Co., it has been estimated[4] that about 40,000,000 people have been treated with this drug in the period of 1950–1970.

This antibiotic is an excellent inhibitor of protein synthesis by bacterial ribosomes.[5] The 50-S subunit appears to be the site of action and chain elongation beyond the first peptide bond is the process affected.

Although the use of chloramphenicol has been questioned recently because of rare cases of aplastic anemia where toxicity has been shown to be dose related,[6] chloramphenicol is still the drug of choice in certain diseases (*e.g.*, typhoid fever).

Chloramphenicol is a particularly interesting drug for modification and structure–activity study. It is well known[4] and it becomes more apparent in the results from this study that it is possible to introduce an interesting variety of structural variation in the side chain and the para position on the phenyl ring without destroying activity. As antibiotics go, chloramphenicol is easy to modify. Certainly it is highly desirable to find a derivative less toxic to bone marrow. Our own interest extends beyond the above in that chloramphenicol seems to be a fine molecule with which to study the use of substituent constants and regression analysis in correlating chemical structure with biological activity and the development of this technique for drug design. Preliminary studies[7-9] were the starting point in the design of the present study. In the present study variation of substituents in the 4 position and in the amide side chain of I have been tested *in vitro* against *Escherichia coli*.



I

Methods

The compounds of Table I were obtained *via* several methods. Those indicated by A under source in Table I were obtained directly from the Parke Davis Co. *N*-Acyl derivatives of D-*threo*-1-(4-nitrophenyl)-2-amino-1,3-propanediol were synthesized by the three different ways developed by Rebstock:[2] (I) amide forma-

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