HI (30 ml) at 60° for 28 hr when no CO_2 was detected in the effluent gases. The clear solution was extracted with $CHCl_3$ (3 × 15 ml) and concentrated *in vacuo* to an oil, which solidified when digested with Et_2O (4 × 30 ml). The product (0.3 g, 39%) was washed with Et_2O several times to yield the analytical sample: mp 104° dec. *Anal.* ($C_5H_8INO_3$) C, N; H: calcd, 3.14; found, 3.62; I: calcd, 49.36; found, 51.75.

Benzyl *N*-Carbobenzoxy-L-2-amino-4-oxo-5-acetoxypentanoate. To a solution of **6** (1.3 g, 3.4 mmol) in Et_2O (80 ml) were added AcOH (4 ml) and Cu powder (0.5 g). The mixture was stirred at 25° for 17 hr. The filtrate from the solids was concentrated *in vacuo* to an oil, which, when triturated with cold Et_2O, yielded the crude 5-acetoxy derivative (0.5 g, 35%). The analytical sample was recrystallized from EtOH–H_2O after treatment with Norit: mp 100–102°. *Anal.* ($C_{22}H_{23}NO_7$) C, H, N.

L-2-Amino-4-oxo-5-acetoxypentanoate (12). A solution of benzyl *N*-carbobenzoxy-L-2-amino-4-oxo-5-acetoxypentanoate (314 mg, 0.76 mmol) in MeOH (50 ml) containing AcOH (0.13 ml) was hydrogenated over 10% Pd–C (0.13 g) for 1.5 hr. The filtrate from the catalyst was concentrated *in vacuo* to give a quantitative yield of **12** (150 mg). The analytical sample was recrystallized from MeOH: mp 137° dec. *Anal.* ($C_7H_{11}NO_5$) C, H, N.

Bis(L-2-amino-4-oxo-5-ylpentanoic acid)phosphine Oxide Hydroiodide (13). A mixture of **6** (2 g, 5.4 mmol), PH_4I (1.7 g), and Et_2O (150 ml) was stirred at 25° under anhydrous conditions for 17 hr. The oily residue after removal of Et_2O *in vacuo* was dissolved in glacial AcOH (60 ml), and PH_4I (2 g) was added. The mixture was stirred at 45–50° for 5 hr and then at 25° for 2 days. The product separated as an orange hygroscopic precipitate and the AcOH supernatant solution was concentrated *in vacuo* to yield additional compound. The product (0.34 g, 29%) was washed successively with small amounts of glacial AcOH and EtOAc to yield the analytical sample: mp 145° dec. *Anal.* ($C_{10}H_{18}IN_2O_7P$) C, H, I, N; P: calcd, 6.67; found, 7.10.

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Synthesis and Antitumor and Antibacterial Activity of Benzoquinones Related to the Mitomycins

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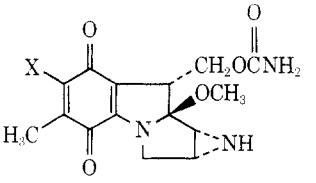
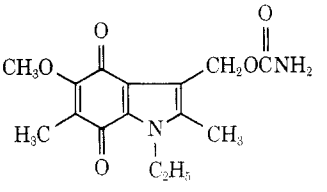
In an attempt to determine the minimum structural requirements for antibacterial and antitumor activity in mitomycin analogs, five different hydroxymethylbenzoquinones were synthesized and converted into their methyl carbamates. The hydroxymethylbenzoquinones were somewhat more active than either their carbamates or simple benzoquinone analogs against certain gram (+) and gram (−) bacteria. In standard NCI screens the carbamates possessed ED_{50} values of 12–26 μg/ml in the KB cell culture and were inactive against L-1210 lymphoid leukemia.

The mitomycins comprise a group of antibiotics whose potent action against both gram-negative and gram-positive bacteria as well as certain tumors is well known. Their usefulness is limited, however, by their relatively high toxicity. In search of less toxic compounds hundreds of derivatives and analogs have been prepared. Although none of these derivatives has proven more useful than mitomycin C (**1a**), both synthetic[1,2] and degradative[3,4] studies have shown certain desaziridinomitosenes to retain some antitumor and antibacterial properties. Related indoloquinones (*e.g.*, **2**) seem to retain only the antibacterial properties.[2,5] Carbazilquinone (**3**), a benzoquinone related to the mitomycins in that it contains the same three carcinostatic functional groups, has been reported to possess a better therapeutic index and higher maximum effectiveness than mitomycin C against lymphoid leukemia L-1210 (see Chart I).[6] It also is effective against both transplantable and primary tumors in mice.[7] The high activity of these partial structures suggested to us that the minimum structural requirements for activity in mitomy-

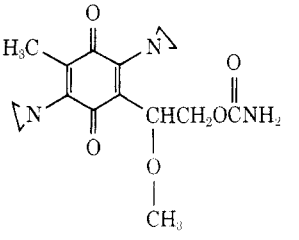
Chart I



1a, X = NH_2 (mitomycin C)
b, X = OCH_3 (mitomycin A)



2

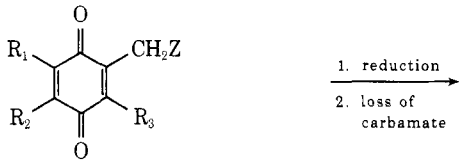


3 (carbazilquinone)

cin analogs might not be defined. Therefore, we initiated a search for the simplest possible active mitomycin analogs.

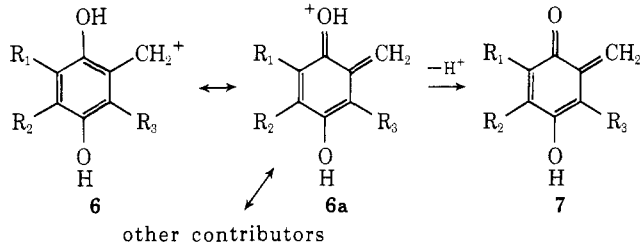
The smallest structural unit capable of being activated *in vivo* by a mechanism similar to that of the mitomycins[8] is a carbamate of a hydroxymethylbenzoquinone with electron-releasing substituents on the ring (**4**). Reduction of such compounds could result in the facile loss of the carbamoyl group forming the highly stabilized carbonium ion **6**. Further stabilization could occur on loss of a proton to form an *o*-quinone methide **7** (Scheme I). Intermediates such as **6** and **7** would react readily with nucleophiles and thus benzoquinones of this type could act as alkylating

Scheme I



4, R_1,R_2, R_3 = H, CH_3, or OCH_3; Z = OCONHR

5, R_1, R_2 = CH_3 or H; R_3 = H, CH_2Cl, CH_2OCOCH_3, or $CH_2OCOOPh$; Z = $OCOCH_3$, OCOOPh, Cl, OCONHEt, or CH_2OH



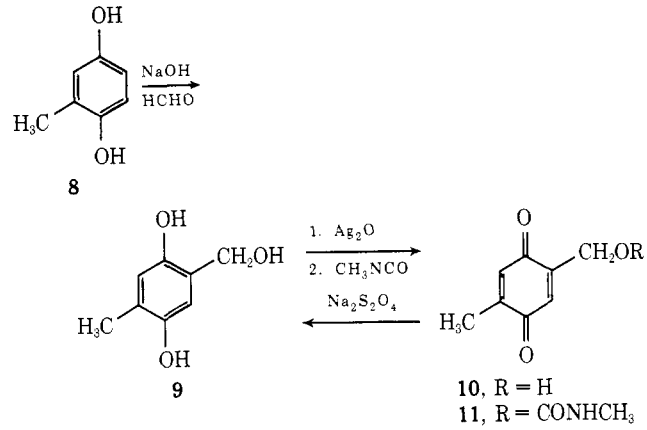
6 **6a** **7**

agents *in vivo*. In view of these possibilities, we undertook the synthesis of a series of suitable benzoquinones with the objective of determining their antitumor and antibacterial activity and relating this to their structures.

During the course of our investigaion, a group at Yale University reported the synthesis and anticancer testing of compounds of type **5**. These compounds were designed according to a rationale similar to ours, and they are closely related in chemical structure. However, only one new compound (**10**) was prepared by both them and us and these two preparations are entirely different. The two investigations are complementary in the sense that our main interest is in carbamates with antibacterial or antitumor activity, whereas their emphasis was on a variety of potential leaving groups, difunctionality, and antitumor activity. Their preliminary data, using the Ad 755 ascites tumor, indicated that the monofunctional compounds are as active as the difunctional ones.[9]

Synthesis. The known hydroxymethyl-1,4-benzoquinone (**27**) was synthesized by an established method[10] from gentisaldehyde. Methyl analog **10** was prepared by hydroxymethylation of toluhydroquinone followed by silver oxide oxidation of the crude intermediate. The instability of intermediate **9** made its direct purification inexpedient. Pure **9** could be produced by sodium dithionite reduction of quinone **10** (Scheme II). Comparison of the melting point of **9** obtained in this manner with those of the known isomeric 3-, 4-, and 6-methyl-2,5-dihydroxybenzyl alcohols[11] fixed the position of hydroxymethylation para to the methyl group. This assignment was supported by the nmr spectrum of **10**. No positional isomers were

Scheme II



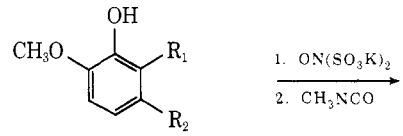
8

9

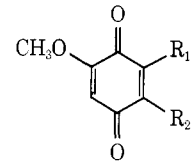
10, R = H
11, R = $CONHCH_3$

isolated from the hydroxymethylation. Compound **10** was independently synthesized using a less direct route by the Yale group.[9] The reduction of 2,4-dimethoxy-3-hydroxybenzaldehyde with $NaBH_4$ produced the corresponding alcohol **14** which was converted directly to the quinone. Oxidation of phenols **12**, **13**, and **14** with potassium nitrosodisulfonate (Fremy's salt) yielded quinones **15**, **17**, and **19**, respectively (Scheme III). These hydroxymethylbenzoquinones were converted into the corresponding methyl carbamates by methyl isocyanate (Table III).

Scheme III



12, R_1 = CH_2OH; R_2 = H
13, R_1 = H; R_2 = CH_2OH
14, R_1 = OCH_3; R_2 = CH_2OH

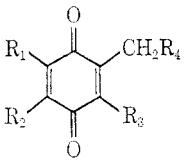


15, R_1 = CH_2OH; R_2 = H
16, R_1 = $CH_2OCONHCH_3$; R_2 = H
17, R_1 = H; R_2 = CH_2OH
18, R_1 = H; R_2 = $CH_2OCONHCH_3$
19, R_1 = OCH_3; R_2 = CH_2OH
20, R_1 = OCH_3; R_2 = $CH_2OCONHCH_3$

The use of $NaBH_4$ to reduce either **22** or **23** gave only polymeric material. High-pressure hydrogenation produced the methylphenols **24** and **25**. Either of these phenols could be oxidized in high yield by Fremy's salt to the corresponding quinones. Aldehyde **26** was formed from **25** using the modified Gatterman reaction (Scheme IV). Its structure was assigned on the basis of the strong hydrogen bonding of the phenolic proton even in dilute solution as determined by its nmr and ir spectra. The product of formylation para to the hydroxyl was not observed. Attempts to reduce **26** to the alcohol and then oxidize to the quinone were unsuccessful.

Biological Activity. The *in vitro* antibacterial activities of the hydroxymethylbenzoquinones and their carbamates are given in Table I. The activities of these compounds, especially **10**, **15**, and **17**, against *Staphylococcus* are greater than the two reference quinones **28** and **29**. The indoloquinone analogs are highly active against resistant

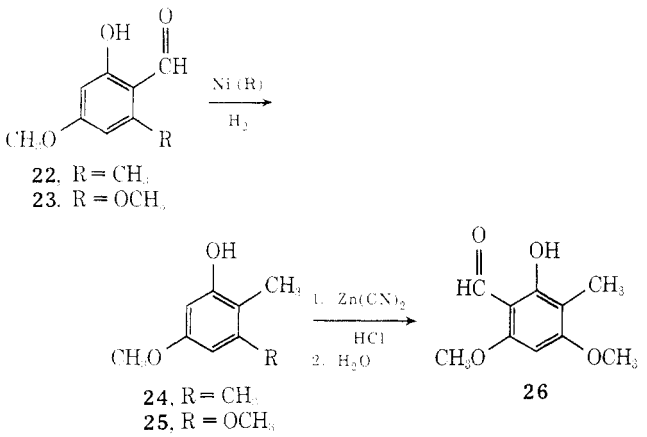
Table I. Antibacterial Activities by the Disk-Plate Assay



No.	R_1	R_2	R_3	R_4	MIC, μg/disk[a] *B. subtilis*[b]	*E. coli*[c]	*Staph. aureus*[d]
27	H	H	H	OH	100	50	50
10	H	CH_3	H	OH	5	50	20
15	OCH_3	H	H	OH	20	100	20
17	H	OCH_3	H	OH	50	50	20
19	H	OCH_3	OCH_3	OH	50	100	100
21	H	H	H	$OCONHCH_3$	50	50	50
11	H	CH_3	H	$OCONHCH_3$	50	50	50
16	OCH_3	H	H	$OCONHCH_3$	50	50	50
18	H	OCH_3	H	$OCONHCH_3$	100	100	50
20	H	OCH_3	OCH_3	$OCONHCH_3$	50	>100	50
28	H	OCH_3	CH_3	H	50	100	>100
29	H	OCH_3	OCH_3	H	50	100	>100
Tetracycline·HCl					0.2	50	50

[a] The smallest quantity in μg which would produce at least a 0.5-mm inhibition zone around a 12.7-mm diameter disk after 24–48 hr of incubation at 37°. Concentrations of 100, 50, 20, 10, 5, etc., were used. [b] *Bacillus subtilis* ATCC 6633. [c] *Esherichia coli* ATCC 14948. [d] *Staphylococcus aureus* strain Smith ATCC 13709.

Scheme IV



22, R = CH_3
23, R = OCH_3
24, R = CH_3
25, R = OCH_3
26

strains of this same bacterium.[2,5] However, against *Bacillus subtilis* all of the compounds were much less active than tetracycline or the indoloquinones.[2] In contrast to other mitomycin analogs,[5] the hydroxymethyl compounds were as active or more active than their carbamate counterparts.

The most active compound **10** and its carbamate **11** were subsequently tested *in vitro* against a more extensive spectrum of bacteria. Representative results of these tests are shown in Table II. Compound **10** has a broad spectrum of antibacterial activity, although it is not highly potent against any bacterium in Table II. Carbamate **11** has only poor activity. These results suggest that instead of a clear minimum structural requirement for antibacterial activity among mitomycin analogs, we have found a gradual and irregular decline in activity as the structures are simplified.

All of the carbamates were screened according to standard protocols by the National Cancer Institute. They bear NSC numbers 166886-90. Against the KB cell culture they showed ED_{50} values of 12–26 μg/ml. In BDF_1 mice they were toxic at doses of 50 mg/kg and higher but inactive against L-1210 lymphoid leukemia at doses of 25 and 12.5 mg/kg. These results contrast with the significant

Table II. Antibacterial Activities by Serial Dilution[a]

Organism	MIC,[b] μg/ml Mitomycin C	**10**	**11**
Diplococcus pneumoniae	0.008	8	125
Streptococcus pyogenes	0.008	8	125
S. aureus Smith	0.03	8	32
S. aureus Smith + 50% serum	0.03	>63	>63
S. aureus Meth. Res.[c]	0.13	32	63
Salmonella enteritidis	0.25	63	>125
Esherichia coli Juhl	1	63	>125
Klebsiella pneumoniae	0.25	63	125
Proteus mirabilis	2	32	125
Pseudomonas aeruginosa	2	>125	>125

[a] We wish to thank T. A. Pursiano and M. Misiek of Bristol Laboratories, Inc., for these data. For a complete description of this test procedure, see M. Misiek, T. A. Pursiano, L. B. Crast, F. Leitner, and K. E. Price, *Antimicrob. Ag. Chemother.*, **1**, 54 (1972). [b] Minimum inhibitory concentration. [c] Methicillin resistant.

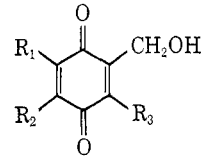
activity reported for closely related benzoquinones against Ad 755 ascites tumor in BDF_1 mice by the Yale group.[9] Therefore, we conclude that minimum structural requirements for antitumor activity in mitomycin analogs have not yet been defined.

Experimental Section

All melting points were determined on a Mel-Temp apparatus and are uncorrected. The structures of all compounds are supported by their ir, nmr, and uv spectra. Solutions were dried ($MgSO_4$) and concentrated under reduced pressure on a rotary evaporator. Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

2-Hydroxymethyl-5-methyl-1,4-benzoquinone (10). Toluhydroquinone (24.8 g, 0.2 mol) and then formaldehyde (32.4 g of 37% solution, 0.4 mol) were dissolved under N_2 in an ice-cold, degassed solution of NaOH (16.0 g, 0.4 mol) in H_2O (184 ml). The resulting solution was stoppered and stored under N_2 at 4° for 72 hr, THF (150 ml) was added, and the mixture was neutralized to pH 7 (AcOH) while at 0°. This solution was extracted three additional times with THF and the combined extracts (300 ml) were

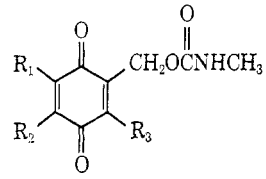
Table III. Synthesis of Hydroxymethylquinones Using Fremy's Salt



No.	R_1	R_2	R_3	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses
17[a]	H	OCH_3	H	78	$CHCl_3$	147–147.5	$C_8H_8O_4$	C, H
15[b]	OCH_3	H	H	64	MeOH	155–155.5	$C_8H_8O_4$	C, H
19	H	OCH_3	OCH_3	70[c]	$CHCl_3$–hexane[d]	90.5–91	$C_9H_{10}O_5$	C, H

[a] From 3-hydroxy-4-methoxybenzyl alcohol: M. Brink, *Acta Univ. Lund., Sect. 2*, **No. 16,** 1 (1965); *Chem. Abstr.*, **64,** 9620*e* (1966). [b] From 2-hydroxy-3-methoxybenzyl alcohol: E. L. Eliel, *J. Amer. Chem. Soc.*, **73,** 43 (1951). [c] Overall yield from 2,4-dimethoxy-3-hydroxybenzaldehyde, which was reduced with $NaBH_4$ in MeOH to the corresponding alcohol. The crude alcohol was then treated directly with Fremy's salt. [d] Crystallization from MeOH yields an unidentified isomer, mp 178–179°.

Table IV. Methyl Carbamates of Hydroxymethylbenzoquinones



No.	R_1	R_2	R_3	CH_3NCO, ml	THF, ml	Rxn time, min	Yield, %	Recrystn solvent	Dec point, °C	Formula	Analyses
21[a]	H	H	H	32	8	300[b]	85	EtOH	115–116	$C_9H_9NO_4$	C, H
11	H	CH_3	H	40		9	77	MeCN	104	$C_{10}H_{11}NO_4$	C, H, N
18	H	OCH_3	H	30	30	360	76	MeCN	114–114.5	$C_{10}H_{11}NO_5$	C, H, N
16	OCH_3	H	H	30	30	60	84	CH_2Cl_2–hexane	106	$C_{10}H_{11}NO_5$	C, H, N
20	H	OCH_3	OCH_3	40		35	71	*c*	112–113	$C_{11}H_{13}NO_6$	C, H, N

[a] From hydroxymethyl-1,4-benzoquinone.[10] [b] At 10°; all the rest are at 25°. [c] The reaction mixture was concentrated and the resulting yellow oil chromatographed over Florisil using CH_2Cl_2–MeCN (10:1) as eluent.

dried overnight. To the filtered extracts Ag_2O (46.4 g, 0.33 mol) and $MgSO_4$ (63.0 g, 0.52 mol) were added and the slurry was stirred in the dark at room temperature for 30 min, filtered, and concentrated. The resulting yellow oil was chromatographed (Florisil,† CH_2Cl_2) to yield a yellow solid (9.1 g) which was crystallized from $CHCl_3$–hexane, mp 78–79° (6.3 g, 21%). This purification process was repeated to give the analytical sample, mp 79.5–80°. *Anal.* ($C_8H_8O_3$) C, H.

2-Hydroxymethyl-5-methyl-1,4-hydroquinone (9). A solution of **10** (0.1 g, 0.66 mmol) in $CHCl_3$ (4 ml) was shaken with a solution of $Na_2S_2O_4$ (0.27 g, 1.55 mmol) and $NaHCO_3$ (0.04 g, 0.48 mmol) in H_2O (2 ml) until colorless. The $CHCl_3$ was removed and EtOAc added. The EtOAc extract was dried and concentrated. The resulting white solid was crystallized (EtOAc–PhH–hexane) to yield 0.04 g, mp 154–155° (lit.[11] 156–157°).

Hydroxymethylquinones of Table III. Solutions of the phenols (0.05 mol) in Me_2CO (200 ml) were poured into stirred solutions of Fremy's salt (potassium nitrosodisulfonate) (26.8 g, 0.10 mol) in 0.9 *M* KH_2PO_4 (1400 ml). Within 5 min at room temperature the color changed from purple to yellow and the solutions were extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried and concentrated to yield yellow solids which were crystallized from the solvents indicated.

Methyl Carbamates of Table IV. Solutions of the hydroxymethylquinones (2.0 g) in mixtures of methyl isocyanate and THF (Table IV) were warmed to boiling, treated with several drops of Et_3N, corked, and allowed to stand at room temperature (**21** at 10°) for 9–360 min. They then were cooled to −15°, filtered, and crystallized from the solvents indicated. The carbamates all decomposed readily; therefore, traces of acid and high temperature had to be avoided in their crystallization.

†Florisil is the trademark of Floridin Co. for a magnesia–silica gel adsorbent.

2,3-Dimethyl-5-methoxyphenol (24). A slurry of 4-methoxy-6-methylsalicylaldehyde[12] (11.0 g, 0.067 mol) and Raney nickel (3 g) in absolute EtOH (75 ml) was rocked at 60° for 2–3 hr under 1800 psi of H_2. After cooling overnight the H_2 was released, the solid removed by filtration, and the solution concentrated to yield a white solid (9.2 g) which was crystallized from $CHCl_3$, mp 91–92° (4.9 g, 49%). The analytical sample (sublimed 0.5 mm, 60°) had mp 93–93.5°. *Anal.* ($C_9H_{12}O_2$) C, H.

3,4-Dimethoxy-2-methylphenol (25) was prepared by the procedure described for **24.** Hydrogenation of 4,6-dimethoxysalicylaldehyde‡ (10.0 g, 0.055 mol) produced a white solid which on crystallization from xylene–hexane had mp 63–64° (lit.[13] mp 60–61°) (7.6 g, 83%).

4,6-Dimethoxy-3-methylsalicylaldehyde (26). Dry HCl was bubbled into a stirred mixture of **25** (5.05 g, 0.03 mol) and $Zn(CN)_2$ (6.15 g, 0.052 mol) in anhydrous Et_2O (70 ml) for 2 hr. Cooling was applied as required to maintain the Et_2O at a gentle reflux. The paste-like precipitate was washed five times with Et_2O, then dissolved in warm H_2O (150 ml), and stirred for 1.5 hr. The resulting slurry was filtered and the solid was dried. It had mp 168–169° (3.8 g, 65%). An analytical sample, recrystallized from Et_2O–Me_2CO, had mp 169–170°. *Anal.* ($C_{10}H_{12}O_4$) C, H. The assignment of this isomer was made primarily on the shift of the phenolic proton in the nmr (TMS), δ 12.10.

Antibacterial Testing. *Staph. aureus* (ATCC 13709), *Escherichia coli* (ATCC 14948), and *B. subtilis* (ATCC 6633) were all maintained on nutrient agar slants. Organisms used for testing were incubated (37°) overnight in nutrient broth or in the case of *B. subtilis* in a tryptone–glucose medium,[14] collected by centrifugation, suspended in 0.5 *M* potassium phosphate buffer (pH 7.5), mixed with "soft" agar (0.7 g of nutrient agar/100 ml of H_2O) at

‡Aldrich Chemical Co., Milwaukee, Wis.

39–40°, and then poured onto solid agar plates. The compounds to be tested were dissolved in the appropriate amount of Me_2CO and aliquots (0.1 ml) of these solutions were placed on paper disks. The disks were dried and placed on the prepared agar plate which was then incubated at 37° for 24–48 hr.

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Synthetic Sweeteners. 3. Aspartyl Dipeptide Esters from L- and D-Alkylglycines

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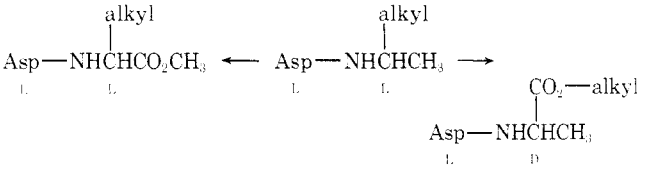
L-Asp-L-Nle-OMe and L-Asp-D-Ala-O(*i*-Pr) are representatives of new series of sweet dipeptides. In the first case, sweetness is obtained with certain side chains of four to six carbons in the C-terminal amino acid and, in the second, with side chains of one to three carbons.

Following the initial discovery of the very pleasant and potent sweet taste of L-aspartyl-L-phenylalanine methyl ester,[1] an intensive investigation of structure–activity relationships was begun. It became clear that L-aspartic acid was essential for sweetness but that considerable latitude was possible in the other part of the molecule. To summarize very briefly, we found that the C-terminal amino acid could be progressively simplified without great loss in sweetness potency. For example, the carbomethoxy group was replaced by methyl or the phenyl by cyclohexyl. Both these changes could be combined to give an amide which was still sweet. The latter result suggested that aliphatic amides of L-aspartic acid might be sweet and this was confirmed.[2] In addition, the methyl ester was always the most potent ester and all the sweet taste resided in the LL isomer for both dipeptides and amides. Table I gives one example of each of the above modifications.

The present work was prompted by two questions. One, could we go from methyl back to carbomethoxy and use unnatural amino acids with long aliphatic side chains and two, could methylene groups in an aliphatic side chain be replaced by carboxyl leading to dipeptide esters of D-amino acids? It was already known that L-Asp-L-Leu-OMe and L-Asp-D-Phe-OMe were bitter so that an affirmative answer to these questions did not seem highly probable.



The methyl esters of L-norleucine, DL-2-amino-5-methylhexanoic acid,[3] DL-2-aminoheptanoic acid,[4] and DL-2-aminooctanoic acid were synthesized and coupled with *N*-carbobenzoxy-L-aspartic acid α-*p*-nitrophenyl ester β-benzyl ester.[5] The resulting protected dipeptides were hydrogenated to give the required dipeptide methyl esters. Physical properties and yields are shown in the tables. It was found that L-Asp-L-Nle-OMe was moderately sweet (40 times sucrose) in contrast to the bitter isomeric L-Asp-L-Leu-OMe. Furthermore, the higher homologs were all sweet with somewhat increased potency. These latter compounds were tasted as a mixture of LL and LD diastereoisomers so that the potency of the taste-bearing isomer could be double the observed value. Based on previous work and on the results with L-Nle, we assume that the sweet taste is associated with the LL isomer but this has not been actually proved.

The first D-amino acid studied was D-alanine. Synthesis of the methyl ester and conversion to L-aspartyl-D-alanine methyl ester followed usual procedures. This compound had a pleasant sweet taste with a potency of 25 times sucrose. Other esters (C_2–C_5) were prepared; maximum sweetness occurred at *n*-propyl (Table II). In subsequent work, isopropyl rather than *n*-propyl esters were used because it was expected that the former would be more stable, thus possibly avoiding the formation of diketopiperazines.

L-Aspartyl dipeptide isopropyl esters were obtained from D-2-aminobutyric acid, D-valine, DL-norvaline, D-alloisoleucine, D-leucine, and D-norleucine. Physical properties of intermediates and final products are shown in the tables. It seems that a small, compact alkyl side chain (CH_3, C_2H_5, i-C_3H_7) is required for high potency, a not unreasonable result since the group is substituting on a receptor site for a methoxycarbonyl group in the LL series.

The problem of explaining the diversity of structure giving a sweet taste has challenged a number of investigators but has as yet no completely satisfactory solution. In-