Bridged Bicyclic and Polycyclic Amino Acids. Potent New Inhibitors of the Fibrinolytic Process

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Several potent new inhibitors of the fibrinolytic process have been designed and synthesized. Starting with the structure–activity relationships for known synthetic fibrinolytic inhibitors, the design of new compounds proceeded on the assumption that an optimum distance between functional carboxyl and amino groups in synthetic amino acids would give maximum antifibrinolytic activity. The desired spacing of amino and carboxyl groups at definite and fixed distances from each other was achieved in a series of bridgehead-substituted amino acids with rigid bicyclic or polycyclic nuclei, such as the bicyclo[2.2.2]octane, bicyclo[2.2.1]heptane, bicyclo[3.2.2]nonane, cubane, or spiro[3.3]heptane nucleus. In particular, a high order of activity was achieved with 4-aminomethylbicyclo[2.2.2]octane-1-carboxylic acid (**7**) and certain of its analogs. This paper reports the synthetic details, biological activities, and structure–activity relationships.

In recent years, there has been considerable interest in the fibrinolytic process and both its activation and inhibition by chemical agents.[1-7] Our interest in inhibitors of fibrinolysis was stimulated by the lack of complete chemical exploration in this area, the desire for more potent and improved agents and the probable clinical utility of an improved agent for the treatment of certain pathological fibrinolytic states.[8]

The structural requirements for known synthetic inhibitors of fibrinolysis have been reported rather extensively, although by no means exhaustively, in the chemical and biological literature.[1,6,7,9-11] At least 3 compounds, all similar amino acids, have received considerable biological and clinical study. These are ε-aminocaproic acid (EACA) (**1**),[8] *p*-aminomethylbenzoic acid (PAMBA) (**2**),[12-16] and *trans*-4-aminomethylcyclohexanecarboxylic acid (AMCA) (**3**).[17-21] These compounds, listed in increasing order of potency, are thought to inhibit fibrinolysis primarily by prevention of the conversion of plasminogen into the proteolytically active enzyme(s) plasmin (fibrinolysin), a process accomplished by natural plasma activators of unknown structure. This natural activation of plasminogen to plasmin can be simulated artificially by the use of the enzymes streptokinase or urokinase.[1,2] In both *in vitro* and *in vivo* tests, the action of streptokinase or urokinase may be inhibited with any of the 3 chemical agents mentioned above. Clinical effectiveness appears to parallel relative potencies of synthetic agents observed in these artificial test systems. The biological data supporting the chemistry and structure–activity relationships which will be discussed below are dependent upon the development of two statistically reliable *in vitro* test procedures which enable rating of the compounds in terms of potency (on a molar basis) relative to a standard of ε-aminocaproic acid (EACA), assigned a value of unity. The two *in vitro* assay procedures employed are identified as methods A and B. These methods are described briefly in the Experimental Section of this paper and in greater detail elsewhere.[22]

The structures and relative antifibrinolytic activities of the known agents mentioned above are listed in Table I, part A.

Factors in the Design of New Agents.—The primary structural requirement for activity appears to be a free amino (or aminomethyl) group and a free CO_2H appropriately separated in a molecule.[1,6,7,9-11] Although the molecule ε-aminocaproic acid (EACA) (**1**) is flexible and can exist in many conformations, it has been known for some time that in a series of homologous unbranched terminal amino acids it exhibits maximum activity.[10] Adding some rigidity to this molecule by incorporation of H_2NCH_2 and CO_2H into the *para* positions of a benzene ring (Table I, **2**) or the 1,4 positions of a cyclohexane ring (**3**) decreases markedly the possible conformations of the molecules and fixes considerably carboxyl-amino distances. Disregarding the change in the acid strength (pK_a) of the CO_2H in **2**, and its possible contribution to activity, this incorporation of the benzene nucleus has increased activity nearly fourfold relative to EACA. Fixing of the same groups in the *trans* diequatorial configuration on the 1 and 4 positions of a cyclohexane ring caused a further increase in activity to a relative potency of 10 (AMCA, **3**); however, the *cis* isomer of this compound, **3a**, is reported to be inactive or much less active than the standard EACA.[10-20]

(1) W. Baumgarten in "Chemical Control of Fibrinolysis," J. Schor, Ed., Wiley, New York, N. Y., in press. W. Baumgarten, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, 22N.

(2) K. N. von Kaulla, "Chemistry of Thrombolysis: Human Fibrinolytic Enzymes," Charles C. Thomas, Springfield, Ill., 1963.

(3) N. K. Sakar, *Proc. Int. Cong. Biochem., 4th, 1958*, **10**, 165 (1959).

(4) S. Okamoto, *Keio J. Med.*, **8**, 211 (1959).

(5) N. Back, Ed., *Ann. N.Y. Acad. Sci.*, **146**, 363 (1968).

(6) S. Müllertz, *Proc. Soc. Exp. Biol. Med.*, **85**, 326 (1954).

(7) A. Sjoerdsma and I. M. Nilsson, *ibid.*, **103**, 533 (1960).

(8) I. M. Nilsson, L. Andersson, and S. E. Björkman, *Acta Med. Scand. Suppl.*, **448**, 1 (1966).

(9) K. Lohmann, F. Markwardt, and H. Landmann, *Thromb. Diath. Haemorrh.*, **10**, 424 (1964).

(10) S. Okamoto, S. Oshiba, H. Mihara, and U. Okamoto, *Ann. N.Y. Acad. Sci.*, **146**, 414 (1968).

(11) L. Skoza, A. O. Tse, M. Semar, and A. J. Johnson, *ibid.*, **146**, 659 (1968).

(12) K. Lohmann, F. Markwardt, and H. Landmann, *Naturwissenschaften*, **50**, 502 (1963).

(13) F. Markwardt, K. O. Haustein, and H. P. Klöcking, *Arch. Int. Pharmacodyn. Ther.*, **152**, 223 (1964).

(14) F. Markwardt and M. Richter, *Acta Biol. Med. Ger.*, **13**, 719 (1964).

(15) F. Markwardt and H. P. Klöcking, *ibid.*, **14**, 519 (1965).

(16) H. Landmann, F. Markwardt, H. G. Kazmirowski, and P. Neuland, *Hoppe-Seyler's Z. Physiol. Chem.*, **348**, 745 (1967).

(17) S. Okamoto, S. Sato, H. Hatano, and U. Okamoto, *Proc. Congr. Int. Soc. Haematol., 9th, 1962*, 265 (1964).

(18) K. Yamamoto and H. Hatano, *Keio J. Med.*, **14**, 99 (1965).

(19) A. H. C. Dubber, G. P. McNicol, and A. S. Douglas, *Brit. J. Haematol.*, **11**, 237 (1965).

(20) B. Melander, G. Glinlecki, B. Granstrand, and G. Hansholf, *Acta Pharmacol. Toxicol.*, **22**, 340 (1965).

(21) L. Andersson, I. M. Nilsson, J. E. Nilehn, U. Hedner, B. Granstrand, and B. Melander, *Scand. J. Haematol.*, **2**, 230 (1965).

(22) W. Baumgarten, L. I. Priester, D. W. Stiller, A. E. W. Duncan, J. L. Ciminera, and L. J. Loeffler, *Thromb. Diath. Haemorrh.*, **22**, 263 (1969).

Comparison of models of **2** and **3** indicates similar H_2N–CO_2H distances for the active compounds but not for the *cis* compound **3a**. For purposes of comparison in these and subsequent compounds, Table I lists distances in ångströms between the CO_2H and the C to which the NH_2 is attached. This distance has been chosen rather than H_2N–CO_2H distances, because in structures such as **3**, rotation of the amino group results in a variable CO_2H–NH_2 distance. The figures in Table I thus represent better an average value for purposes of comparing one molecule with another. As indicated in Table I, this distance is 5.87 Å for **2**, 5.82 Å for **3**, and 4.6 Å for the *cis* isomer of **3** (**3a**). Since **3a** was inactive and **1** through **3** exhibited activity, and since it was known, along with other structure–activity relationships, that N substitution or esterification of CO_2H eliminates activity, at least *in vitro*, we proceeded on the assumption that an optimum distance between free NH_2 and free CO_2H in active synthetic amino acids might not yet have been achieved; that is, we assumed, as have others,[11] that NH_2 and CO_2H in an active structure must approach or interact favorably with a hypothetical receptor molecule of an unknown nature in order to exert an inhibitory effect. Therefore, an ideal structural type for a study of the influence of structural variations on biological activity should permit (1) a systematic variation of the H_2N–CO_2H distance, (2) the fixation of this distance by structural rigidity, (3) the variation of this distance with minimum change in the central portion of the structure holding the NH_2 and CO_2H groups, and (4) the approach of these groups to a receptor with minimum steric hindrance.

Some of these conditions and goals were fulfilled through synthesis of a series of amino acids based upon rigid bicyclic or polycyclic nuclei, in particular the bicyclo[2.2.1]heptanes, bicyclo[2.2.2]octanes, bicyclo[3.2.2]nonanes, cubanes, and spiro[3.3]heptanes. Structures synthesized are listed in part B of Table I. Relative potency figures speak for the success of this approach. Six new amino acids have an *in vitro* potency equal to or greater than the *trans* isomer of 4-aminomethylcyclohexanecarboxylic acid (AMCA, **3**), the most effective fibrinolytic inhibitor previously reported.

Structure–Activity Relationships.—An interesting group of three novel bicyclic amino acids resulted from the addition of a C_1, C_2, or C_3 bridge across the 1,4 positions of the cyclohexane ring of **3**, locking the ring into a boat conformation. The resulting rigid and symmetrical molecules **4**, **5**, and **7**, with bridgehead substituents protruding from colinear or nearly colinear bonds, enabled variation of functional group distances over a range somewhat shorter than that observed in the cyclohexane and aromatic compounds. The distances are 5.34 Å for 4-aminomethylbicyclo[2.2.1]heptane-1-carboxylic acid (**4**), 5.67 Å for the bicyclo[2.2.2]octane analog (**7**), and 5.76 Å for the bicyclo[3.2.2]nonane analog (**5**). Resulting activities measured *in vitro* were 5, 103, and 19, respectively.

The highly active compound 4-aminomethylbicyclo[2.2.2]octane-1-carboxylic acid (**7**) presents functional groups fixed in space by a highly symmetrical molecule which would appear identical in approach to a hypothetical receptor molecule or surface from all three sides or bridges. The bicyclo[3.2.2]nonane derivative **5** spreads functional groups slightly apart relative to **7**, the bonds at the bridgehead positions are no longer colinear, and an extra C in one bridge may offer some steric hindrance to receptor interaction not seen in **7**. These factors may account for the decreased activity observed for **5**. In the case of the bicyclo[2.2.1]heptane derivative **4**, the distance between functional groups is shortened as indicated; moreover, bonds at the bridgehead positions are again not colinear, but bent slightly upward, resulting in variable CO_2H–NH_2 distances as these groups are rotated. Apparently in the bicycloheptane **4**, the considerably reduced distance between functional groups is much shorter than required for the proper receptor fit. It appears that in this series of three bridged cyclohexanes, the optimum distance is at least most closely approached if not achieved exactly in the bicyclooctane **7**.

Two compounds were prepared in which the H_2NCH_2 group of **5** or **7** is replaced by NH_2 attached directly to the bridgehead position; *i.e.*, 5-aminobicyclo[3.2.2]nonane-1-carboxylic acid (**6**) and 4-aminobicyclo[2.2.2]octane-1-carboxylic acid (**12**). Compound **12** has been reported previously by Roberts, *et al.*[23] Both compounds were found to be inactive, an observation which may result from the greatly shortened distances involved (4.22 and 4.13 Å). However, an examination of models also indicates that a bridgehead NH_2 group which protrudes straight out from the bicyclic nucleus would in addition be severely hindered in attachment to a receptor surface if the CO_2H were associated with this surface in some manner at the same time. In contrast, H_2NCH_2 substitution can allow greater flexibility and freedom to associate with a receptor molecule.

It might be noted here that similar shortening of the C chain in unbranched amino acids (or in *p*-aminobenzoic acid or 4-aminocyclohexanecarboxylic acid) has also been reported to decrease or eliminate activity.[9] However, in the aromatic series, the basicity of the NH_2 group is completely altered by this change and in the cyclohexane series, one can not be certain about the isomeric purity of the material tested.

Because of the high activity of 4-aminomethylbicyclo[2.2.2]octane-1-carboxylic acid (**7**), several structural variations are of special interest. Introduction of a double bond, producing the bicyclo[2.2.2]octene (**8**), was found to decrease activity to 37. This perhaps may be rationalized by a slight distortion of the nucleus by the double bond, spreading of the bridgehead C atoms and slight lengthening of the distances between functional groups. This results in an NH_2–*C* to *C*O_2H distance of 5.76 Å and is based upon calculations reported in 1967 by Baker, *et al.*, for an untwisted bridge structure.[24] Also, the slightly increased protrusion of the two resulting ethylenic protons on one side of the hydrocarbon sphere may account for reduction in activity; however, of more importance perhaps is the resulting alteration in p*K* of the CO_2H. Observed p*K* values were 3.6 and 9.7 for the unsaturated amino acid **8** and 4.6 and 9.9 for the saturated amino acid **7**; that is, unsaturation decreased the p*K* of the CO_2H by roughly 1 p*K* unit. Bulky substituents at the 2 and 5 positions

(23) J. D. Roberts, W. T. Moreland, Jr., and W. Frazer, *J. Amer. Chem. Soc.*, **75**, 637 (1953).

(24) F. W. Baker, R. C. Parish, and L. M. Stock, *ibid.*, **89**, 5677 (1967).

TABLE I
FIBRINOLYTIC INHIBITORS

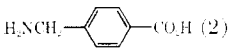
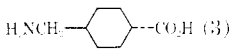
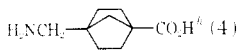
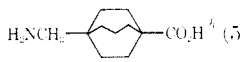
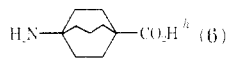
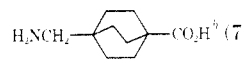
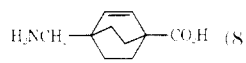
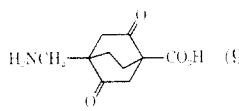
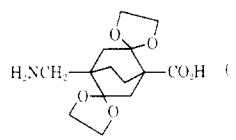
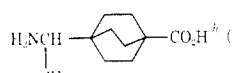
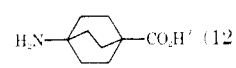
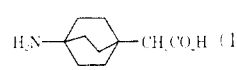
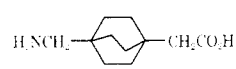
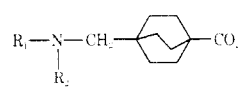
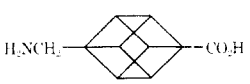
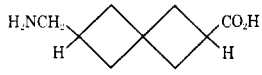
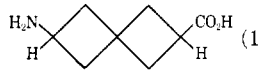
A. Reference inhibitors	Relative activities[a] (*in vitro*) — Method A[b]	Method B[c]	Distance[d]	p*K*[e]
$H_2N(CH_2)_5COOH$ (1) (EACA)	1	1	6.3[f]	4.4 10.3
 (2) (PAMBA)	3.7	3.7	5.87	3.9 9.2
 (3) (AMCA)	10.3	10.5	5.82	4.3 10.2
cis isomer of 3 (3a)	<0.1[g]		4.6	
B. New inhibitors				
Bicyclo[2.2.1]heptane derivatives				
 (4)	5		5.34	4.9 9.9
Bicyclo[3.2.2]nonane derivatives				
 (5)	19		5.76	4.8 10.0
 (6)	0		4.22	4.5 10.3
Bicyclo[2.2.2]octane derivatives				
 (7)	103	68	5.67	4.6 9.9
 (8)	37		5.76	3.6 9.7
 (9)	15		~5.67	3.8 8.7
 (10)	0		5.67	
 (11)	78		5.67	4.5 10.2
 (12)	0		4.13	4.4 9.6
 (13)	0		4.8	
 (14)	17		~6.4	4.5 10.1
 (15)				
R_1	R_2	R_3		
CH_3CO (a)	H	H	0	
C_6H_5CO (b)	H	H	0	
$CH_3C_6H_4SO_2$ (c)	H	H	0	
$CH_3C_6H_4SO_2$ (d)	CH_3	H	0	
H (e)	CH_3	H	0	
H (f)	H	CH_3	0	
H (g)	H	$CH_2C_6H_5$	0	
Cubane derivatives				
 (16)	5		5.76	

TABLE I (*Continued*)

B. New inhibitors	—Relative activities[a] (*in vitro*)— Method A[b]	Method B[c]	Distance[d]	p*K*[e]
Spiro[3.3]heptane derivatives				
 (17)	0		~6.3	4.4 10.2
 (18)	0		~5.3	4.3 9.9

[a] Relative activities are assigned on a molar basis with the activity of EACA given a value of 1.0. [b] The inhibition of clot lysis produced by streptokinase-activated human plasminogen.[22] [c] The inhibition of lysis of a preformed plasma clot induced by *o*-thymotic acid.[22] [d] Distance in ångströms between the CO_2H and the C to which the NH_2 group is attached, calculated or measured on models. [e] Approximate values determined by potentiometric titration in aq solution. [f] This distance is variable; distance for the most extended form is given. [g] Value for *cis* isomer taken from the literature, see ref 10, 21. [h] Hydrochloride used in biological assays. [i] See ref 23.

of **7**, such as observed in 4-aminomethylbicyclo[2.2.2]-octa-2,5-dione-1-carboxylic acid (**9**) or its bisketal **10** were found to reduce activity to 15 in the former and 0 in the latter compound. Steric factors are probably most important; however, it should be noted that the introduction of free keto groups in **9** results also in a sizeable change in p*K*'s of the molecule (p*K*'s of 3.8 and 8.7 for **9** and 4.6 and 9.9 for **7**).

Branching of the 4-CH_2 group of **7**, such as in 4-(α-aminoethyl)bicyclo[2.2.2]octane-1-carboxylic acid (**11**) interestingly had only a slight diminishing effect on the activity. The observed relative potency for **11** was 78. Apparently, this extra Me has little influence on the ability of an amino group to reach a receptor surface. We have observed also that similar substitution of *p*-aminomethylbenzoic acid (**2**) to give *p*-(α-aminoethyl)benzoic acid decreased activity from 3.7 to 2.5 while a comparable Me substituent in EACA (**1**) to give 6-aminoheptanoic acid appeared to abolish activity. This diminishing effect of Me branching thus appears to be general.

Compounds **13** and **14**, where a CH_2 is inserted between the CO_2H and the bicyclic nucleus, represent parallels to similar variations reported by Lohmann, *et al.*,[9] in the aromatic and cyclohexane series. The interesting isomer of the highly active **7**, that is, 4-aminobicyclo[2.2.2]octane-1-acetic acid (**13**), was found to be inactive, most probably for reasons mentioned above involving steric hindrance around the bridgehead NH_2. In other series, it has been reported that *p*-aminophenylacetic acid is inactive and 4-aminocyclohexane-1-acetic acid is much less active than its 4-aminomethyl analog.[9] However, comparison again involves a complete change in the basicity of the NH_2 in the aromatic series and an uncertainty about isomeric purity in the cyclohexane series. 4-Aminomethylbicyclo[2.2.2]octane-1-acetic acid (**14**), found to have a relative potency of 17, again appears to parallel reported literature observations in the aromatic and cyclohexane series. In the aromatic series, *p*-aminomethylphenylacetic acid was found to be active but less so than *p*-aminomethylbenzoic acid, and in the cyclohexane series, 4-aminomethylcyclohexaneacetic acid, of uncertain isomer composition, is reported to be active, but less so than *trans*-4-aminomethylcyclohexanecarboxylic acid (**3**).[9] In all series, increased distances between functional groups and the greater number of possible conformations probably are important factors in determining observed activity.

Other authors have observed previously that substitution of an amino group or esterification of a carboxyl group in amino acids eliminates *in vitro* antifibrinolytic activity. Similarly, we have found that N-acylation, N-methylation, and esterification of **7** all eliminated *in vitro* activity completely (**15a** through **15g**).

Two other types of molecules were investigated as "supports" for NH_2 and CO_2H groups as discussed above: (1) the cubane system, and (2) the spiro[3.3]-heptane system. We had observed, as have others, that C–C distances diagonally across the cubane structure are very similar to those between bridgehead C's in bicyclo[3.2.2]nonane or bicyclo[2.2.2]octane. For this reason, the novel amino acid 4-aminomethylcubane-1-carboxylic acid (**16**) was synthesized, giving a CO_2H–NH_2CH_2 distance of 5.76 Å, equal to that in the bicyclo[3.2.2]nonane **5** and slightly greater than that observed in the bicyclo[2.2.2]octane **7**, (5.67 Å). The observed relative activity of the cubane amino acid was 5, supporting again with an active compound the original concept of structural design. It is possible that the lower activity observed as compared, for example, with the bicyclo[3.2.2]nonane **5** may be a consequence of the protons protruding from the corners of the cube, giving greater steric hindrance to approach to a receptor than observed with bicyclic compounds. Two other compounds, 6-aminomethylspiro[3.3]heptane-2-carboxylic acid (**17**) and 6-aminospiro[3.3]heptane-2-carboxylic acid (**18**) were found to be inactive. The distances involved (6.3 and 5.3 Å, respectively) appear to be considerably longer and shorter, respectively, than those found in active compounds; models also reveal considerably different steric and asymmetric features in the central hydrocarbon portion of these molecules.

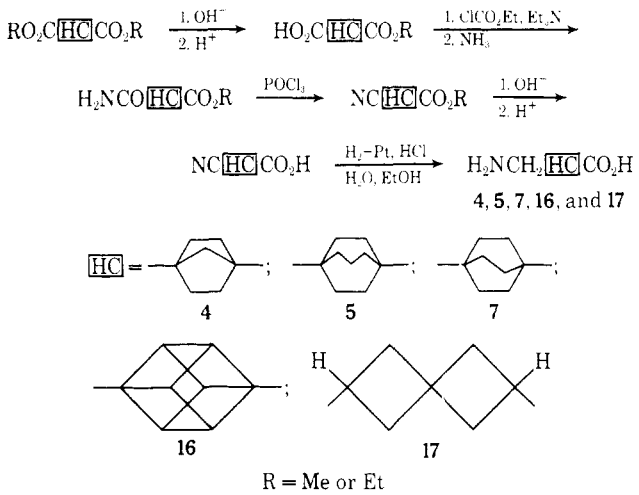
In summary, the approach described has produced novel amino acids possessing *in vitro* antifibrinolytic activity equal to or greater than that of known fibrinolytic inhibitors. 4-Aminomethylbicyclo[2.2.2]octane-1-carboxylic acid (**7**), the most active of these, appears also to be a considerably more potent *in vivo* fibrinolytic inhibitor than those previously available. It appears to be well absorbed orally, is relatively nontoxic, and free of other undesirable pharmacological action. Preliminary biological work is described in another publication.[22]

Syntheses.—The preparation of the bridged amino acids listed in Table I involves multistep syntheses in

all cases. The synthetic routes are shown in Schemes I through VI. Certain of the required intermediates have been described in the literature as cited in the Experimental Section.

The saturated aminomethyl acids, **4**, **5**, **7**, **16**, and **17** were prepared from the corresponding known symmetrical diesters, as indicated in Scheme I. Conditions em-

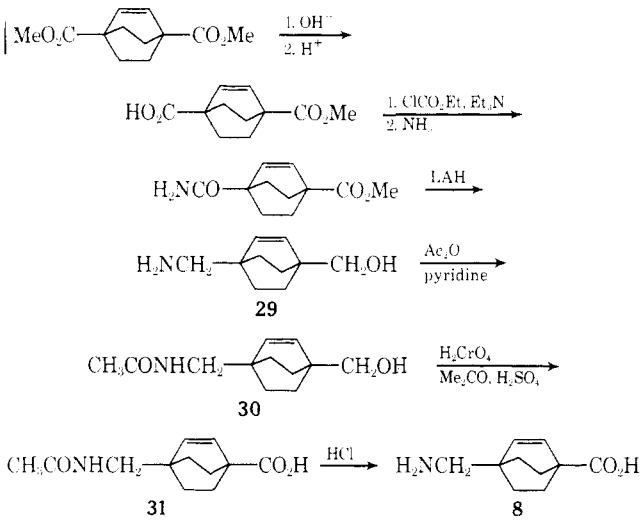
SCHEME I



ployed in all but the last step were patterned after those originally reported by Roberts, *et al.*, for the syntheses of 4-cyanobicyclo[2.2.2]octane-1-carboxylic acid.[23]

Preparation of the unsaturated analog of **7**, 4-aminomethylbicyclo[2.2.2]oct-2-ene-1-carboxylic acid (**8**), involved the synthetic scheme outlined in Scheme II.

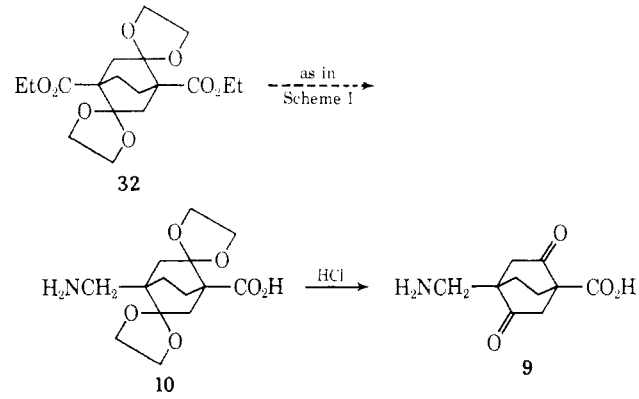
SCHEME II



starting with the unsaturated diester dimethyl bicyclo[2.2.2]oct-2-ene-1,4-dicarboxylate prepared according to the method of Kauer,[25] and proceeding through methyl 4-carbamoylbicyclo[2.2.2]oct-2-ene-1-carboxylate subsequently reported by Baker and Stock.[26]

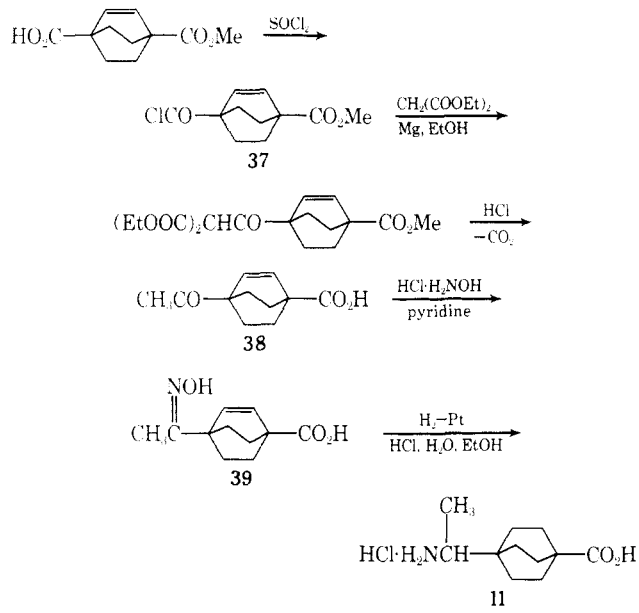
4-Aminomethylbicyclo[2.2.2]octa-2,5-dione-1-carboxylic acid (**9**) was prepared essentially as outlined in Scheme I, starting from the known diethyl 2,5-bisethylenedioxobicyclo[2.2.2]octane-1,4-dicarboxylate (**32**), and after conversion into the amino acid **10**, final acid removal of the 2,5-ketal protecting groups (see Scheme III).

SCHEME III



Preparation of the α-branched homolog of **7**, 4-(α-aminoethyl)bicyclo[2.2.2]octane-1-carboxylic acid (**11**), was accomplished as indicated in Scheme IV.

SCHEME IV



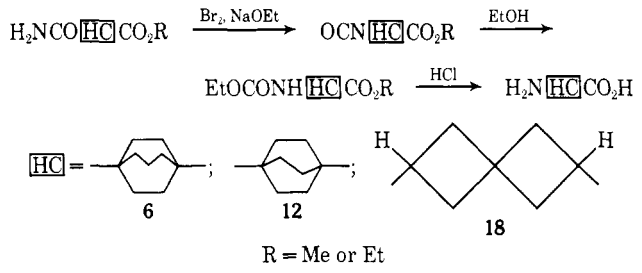
The three lower homologs which lack the CH_2 of **5**, **7**, and **17**, specifically **6**, **12**, and **18**, were prepared by Hofmann degradation of amido ester intermediates according to the method used by Roberts, *et al.*,[23] for the earlier preparation of **12**. This involves production of the isocyanate and ethyl carbamate from the primary amide, followed by final hydrolysis of the carbamate to the desired amino acid (Scheme V).

Scheme VI outlines the similar procedures used for the preparation of 4-aminobicyclo[2.2.2]octane-1-acetic acid (**13**) and 4-aminomethylbicyclo[2.2.2]octane-1-acetic acid (**14**) from their respective lower homologs **12** and **7**. Amino acids **12** and **7** were first acylated for N-protection, then the free CO_2H converted into diazoketo functions by the action of $SOCl_2$ followed by CH_2N_2. Curtius reaction of diazo ketones to give iso-

(25) J. C. Kauer, R. E. Benson, and G. W. Parshall, *J. Org. Chem.*, **30**, 1431 (1965).

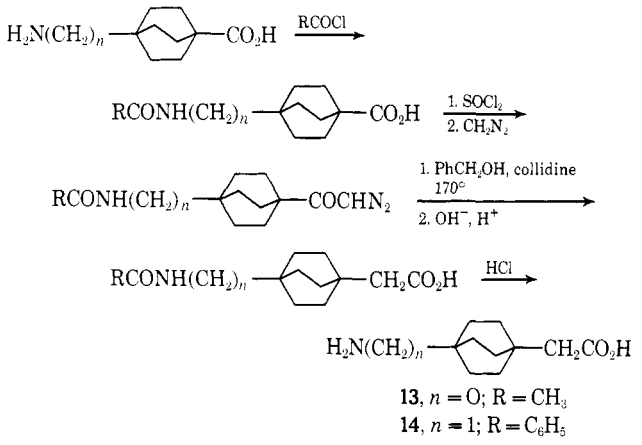
(26) F. W. Baker and L. M. Stock, *ibid.*, **32**, 3344 (1967).

Scheme V



cyanates and benzyl esters was accomplished at 170° with collidine in $PhCH_2OH$. Final base, then acid hydrolysis produced the free amino acids **13** or **14**.

Scheme VI



N-Me, *N*-acyl, or ester derivatives of 4-aminomethylbicyclo[2.2.2]octane-1-carboxylic acid (**7**) (**15a** through **15g**, Table I) were prepared directly from the parent amino acid **7** by well-established literature procedures employed with many other amino acids.

Experimental Section

All melting points (uncorrected) were determined using a Uni-Melt Thomas-Hoover melting point apparatus. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of theoretical values. Nmr spectra were determined on a Varian A60-A Spectrometer and ir spectra on a Perkin-Elmer Model 11 MS recording spectrophotometer.

I. 4-Aminomethylbicyclo[2.2.1]heptane-1-carboxylic Acid (4) (See Scheme I). 4-Cyanobicyclo[2.2.1]heptane-1-carboxylic acid (19) was prepared in 8 steps essentially according to the procedure reported by Wilcox and Leung.[27]

4-Aminomethylbicyclo[2.2.1]heptane-1-carboxylic Acid Hydrochloride (4).—To 165 mg (1.0 mmole) of 4-cyanobicyclo[2.2.1]-heptane-1-carboxylic acid (**19**) dissolved in 20 ml of EtOH was added 8 ml of H_2O, 2.0 ml of 1.00 *N* HCl, and 100 mg of PtO_2 catalyst (Engelhard 85%). The mixture was hydrogenated at 2.46 kg/cm² and room temp for 1 hr. After removal of the catalyst by filtration through sintered glass, the filtrate was evaporated *in vacuo* at steam bath temperature, then 3 times more with fresh portions of 95% EtOH. A white solid remained, mp 255–258° dec. The material was recrystallized 3 times from 95% EtOH–Et_2O to give an analytical sample: mp 256–258° (185 mg, 90%); tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.38 (ninhydrin, red or I_2); ir (KBr) 1690 (COOH), 1560, and 1510 cm^{-1} (NH_3^+); nmr (D_2O) δ 1.0–2.2, sharp central peak at 1.58 (10, bicyclic ring), 3.14 (2, CH_2N), and 4.57 ppm (4, COOH and NH_3^+ exchangeable protons). *Anal.* ($C_9H_{15}NO_2 \cdot HCl$) C, H, Cl, N.

II. 5-Aminomethylbicyclo[3.2.2]nonane-1-carboxylic Acid (5) (See Scheme I). Diethyl 6,8-dioxobicyclo[3.2.2]nonane-1,5-dicarboxylate (20) was prepared essentially as described in the literature.[28] Before use, the product was dried overnight at 50° *in vacuo*, mp 130.5–133° (reported,[28] 132°).

Diethyl 6,8-bis(ethylenedithio)bicyclo[3.2.2]nonane-1,5-dicarboxylate (21) was prepared from the diketo diester **20**, 1,2-ethanedithiol, and BF_3–Et_2O according to the procedure employed by Roberts, *et al.*,[23] for the analogous bicyclo[2.2.2]octane derivative. The product, obtained in 63% yield, was recrystallized from abs EtOH, then from hexane; needles, mp 101–103.5°. *Anal.* ($C_{19}H_{28}O_4S_4$) H, S; C: calcd, 50.86; found, 51.47.

Diethyl bicyclo[3.2.2]nonane-1,5-dicarboxylate (22) was prepared in approximately 80% yield by desulfurization of the bisthioketal **21** in refluxing EtOH with active Raney Ni, again following a procedure described by Roberts, *et al.*,[23] for the bicyclo-[2.2.2]octane series. The product was a colorless liquid, bp 142–145° (0.8 mm). *Anal.* ($C_{15}H_{24}O_4$) H; C: calcd, 67.13; found, 68.04. Glc of the analytical material indicated the presence of a minor contaminant (5% or less, not removable by distillation). However, this material proved of purity sufficient for use in the preparation of **23**.

Ethyl hydrogen bicyclo[3.2.2]nonane-1,5-dicarboxylate (23) was obtained in 62% yield by saponification of the diester **22** with 1 mole of NaOH in aq EtOH, as described by Roberts, *et al.*,[23] for the corresponding bicyclo[2.2.2]octane compounds. In addition, 16% of the starting diester was recovered by extraction with Et_2O from a basic aq layer. The desired product was separated from a small amount of the corresponding diacid (6.5% recovered) by column chromatography on silica gel ($CHCl_3$ eluent). The desired monoacid monoester **23** was recrystallized from hexane (Dry Ice–Me_2CO), then sublimed, mp 53.5–55°. *Anal.* ($C_{13}H_{20}O_4$) H; C: calcd, 64.98; found, 65.41.

Ethyl 5-carbamoylbicyclo[3.2.2]nonane-1-carboxylate (24) was prepared from the monoacid monoester **23** by the mixed anhydride procedure (ethyl chloroformate and anhydrous NH_3) developed by Roberts, *et al.*,[23] for corresponding compounds in the bicyclo[2.2.2]octane series (Scheme I). The amide ester **24**, mp 83.5–85°, was obtained in 71.6% yield (needles from C_6H_6–hexane). *Anal.* ($C_{13}H_{21}NO_3$) C, H, N.

Ethyl 5-cyanobicyclo[3.2.2]nonane-1-carboxylate (25), an oil, was prepared in 86% yield by dehydration of the amide ester **24** with $POCl_3$ in $ClCH_2CH_2Cl$, according to the method employed by Roberts, *et al.*,[23] in the bicyclo[2.2.2]octane series (Scheme I). The material was shown to be homogeneous by tlc, possessed the expected ir absorptions, and was saponified directly without further purification.

5-Cyanobicyclo[3.2.2]nonane-1-carboxylic acid (26) was obtained in 87% yield by saponification of the cyano ester **25**, again using the procedure of Roberts, *et al.*,[23] (Scheme I). The product was recrystallized from C_6H_6 for analysis, mp 186–188°. *Anal.* ($C_{11}H_{15}NO_2$) C, H, N.

5-Aminomethylbicyclo[3.2.2]nonane-1-carboxylic Acid Hydrochloride (5).—The cyano acid **26** was reduced over Pt in aq EtOH containing HCl as described above for the production of **4**. The crude white hydrochloride (**5**), mp 286–289° was recrystallized from 95% EtOH–Et_2O: mp 291.5–293° dec (yield, 81%); tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.69 (ninhydrin, pink); nmr (D_2O) δ 1.3–2.2 with sharp peaks at 1.73, 1.80, and 1.95 (14, bridged ring), and 2.87 ppm (s, 2, CH_2N); equiv wt calcd 233.75, found 234.33; pK_1 4.8, pK_2 10.0 (H_2O). *Anal.* ($C_{11}H_{19}NO_2 \cdot HCl$) C, H, Cl, N.

III. 5-Aminobicyclo[3.2.2]nonane-1-carboxylic Acid (6) (See Scheme V). Ethyl 5-(*N*-carboethoxy)aminobicyclo[3.2.2]nonane-1-carboxylate (27) was prepared in 83% crude yield from the amide ester **24**, following the Hofmann degradation procedure employed by Roberts, *et al.*,[23] in the bicyclo[2.2.2]octane series (see Scheme V). The urethane ester **27**, a liquid, tlc (sil G) 9:1, C_6H_6–MeOH, R_f 0.9, was hydrolyzed to the amino acid without further purification.

5-Aminobicyclo[3.2.2]nonane-1-carboxylic Acid (6).—To 0.93 g (3.28 mmoles) of crude **27** was added 20 ml of concd HCl and the mixture refluxed for 8 hr. Filtration removed a small quantity of amorphous solid. Evaporation *in vacuo* gave a pale yellow solid, which was recrystallized from 95% EtOH–Et_2O, giving a crystalline hydrochloride, mp 259–261° (0.42 g, 58%). Because of difficulty in removing one minor impurity, the material was converted into the free amino acid by passage of a solution in H_2O through a column of Dowex-1 acetate. The free amino acid

(27) C. F. Wilcox, Jr., and C. Leung, *ibid.*, **33**, 877 (1968).

(28) P. C. Guha, *Chem. Ber.*, **72**, 1359 (1939).

was recrystallized from aq Me_2CO: wt 253 mg; mp >360°; tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.58 (ninhydrin, yellow); nmr (D_2O, CF_3COOH) δ 1.6–2.1 ppm with sharp peaks at 1.81 and 1.90 ppm (ring protons); equiv wt calcd 183.25, found 184.32; pK_1 4.5, pK_2 10.3 (H_2O). *Anal.* ($C_{10}H_{17}NO_2$) C, H, N.

IV. 4-Aminomethylbicyclo[2.2.2]octane-1-carboxylic Acid (7) (See Scheme I). A. Hydrochloride.—Reduction of 4-cyanobicyclo[2.2.2]octane-1-carboxylic acid (**28**), prepared as described by Roberts, *et al.*,[23] was accomplished as described above for **4**. The crude amino acid hydrochloride was purified by dissolution in hot 95% EtOH and reprecipitation with Et_2O. The white solid, mp 318–319° dec (placed in sealed capillary at 250°) after air drying, was obtained in 90.5% yield. Three recrystallizations from 95% EtOH–Et_2O gave analytically pure material: mp 318–319° dec, which was dried at 110° over P_2O_5 for 18 hr at 0.08 mm; tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.65 (ninhydrin, pink); nmr (D_2O) δ 1.3–2.0 with sharp absorptions at 1.62 and 1.72 (12, bridged ring) and 2.81 ppm (s, 2, CH_2N); equiv wt calcd 219.72, found 219.87; pK_1 4.6, pK_2 9.9 (H_2O). *Anal.* ($C_{10}H_{17}NO_2 \cdot HCl$) C, H, Cl, N.

B. Free Amino Acid.—Analytically pure hydrochloride (300 mg) was dissolved in 2 ml of distilled H_2O, placed above a column of H_2O-washed Dowex-1 acetate (20 g wet weight) and eluted with distilled H_2O. The first few milliliters of eluent, when evaporated *in vacuo* (60–70°), afforded the free amino acid. After 1–3 recrystallizations from H_2O–Me_2CO, the air-dried material, or material dried at 110° for 24 hr, melted at 269–274° dec and some darkening and softening at 265° (placed in sealed capillary at 180°), and the material analyzed slightly low in C. Redrying at 137° for 24 hr at 0.35 mm over P_2O_5 raised the melting point to 280–283° dec (softening at 274–275°). *Anal.* ($C_{10}H_{17}NO_2$) C, H, N.

V. 4-Aminobicyclo[2.2.2]octane-1-carboxylic acid (12) was prepared as described previously by Roberts, *et al.*[23] (see Scheme V).

VI. 4-Aminomethylbicyclo[2.2.2]oct-2-ene-1-carboxylic Acid (8) (See Scheme II). 1-Aminomethyl-4-hydroxymethylbicyclo-[2.2.2]oct-2-ene (29).—To 1.52 g (40 mmoles) of LAH in 50 ml of dry THF at reflux was added dropwise with stirring a solution of 1.90 g (9.10 mmoles) of methyl 4-carbamoylbicyclo[2.2.2]oct-2-ene-1-carboxylate.[26] After the addition was completed (1 hr), the mixture was stirred and refluxed for 10 hr. Excess LAH was decomposed by the cautious addition of H_2O, the THF removed *in vacuo*, and the aq solution basified with KOH and extracted continuously with Et_2O. Drying of the Et_2O extracts ($MgSO_4$), filtration, and removal of the Et_2O *in vacuo* left a basic colorless viscous oil, 0.85 g (56%). After removal of solvents *in vacuo* the material was used directly for N-acetylation without further purification.

1-Acetamidomethyl-4-hydroxymethylbicyclo[2.2.2]oct-2-ene (30).—To 0.85 g (5.10 mmoles) of **29** dissolved in 15 ml of dry pryidine was added over 30 min 0.46 g (4.50 mmoles) of Ac_2O in 10 ml of pyridine. The mixture was stirred overnight at room temperature. After removal of the pyridine *in vacuo*, the remaining oil was taken up in 50 ml of EtOAc, extracted twice with 5 ml of 3 *N* HCl and twice with 5 ml of saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and stripped to an oil which quickly solidified. Recrystallization from MeCN removed a trace of *N*,*O*-diacetyl derivative, giving the crystalline *N*-acetyl compound: mp 137–139° (594 mg, 56%); ir (KBr) 1650, 3270 (CONH) and 3350–3400 cm^{-1} (OH); nmr ($CDCl_3$) δ 1.37 (m, broad, 8, ring CH_2), 2.00 (s, 3, CH_3CO), 3.34 (d, J = 6 Hz, 2, CH_2N), 3.61 (s, 2, CH_2O), 5.8 (broad, 1, NH), and 6.18 ppm (q, J = 9 Hz, 2, CH=CH).

4-Acetamidomethylbicyclo[2.2.2]oct-2-ene-1-carboxylic Acid (31).—To 209 mg (1.0 mmole) of **30** dissolved in 15 ml of Me_2CO at 10° was added 0.60 ml of 2.67 *M* Jones reagent in 0.10-ml portions over a period of 15 min along with 30 ml of Me_2CO, keeping the temperature between 10 and 15°. After stirring an additional 30 min at this temperature, 2 drops of *i*-PrOH were added to destroy the excess oxidant. Finally, 50 ml of H_2O was added to dissolve the precipitated salts. After removal of the Me_2CO *in vacuo*, the product was recovered by continuous extraction of the aq layer with Et_2O. After drying ($MgSO_4$), evaporation of the Et_2O left the amide acid **31**. Recrystallization from MeCN gave the product as needles: mp 220–225°; nmr (CF_3COOH) δ 1.2–2.2 (m, complex, 8, ring CH_2), 2.58 (s, 3, CH_3CO), 3.73 (d, J = 6 Hz, 2, CH_2N), 6.23 and 6.70 (d's, J = 8.5 Hz, 2, AB pattern for CH=CH), and 8.7 ppm (s, broad, 1, NH); ir (KBr) 1650 (CONH) and 1690 cm^{-1} (CO_2H).

4-Aminomethylbicyclo[2.2.2]oct-2-ene-1-carboxylic Acid (8).—To 183 mg (0.82 mmole) of **31** was added 20 ml of EtOH and 40 ml of 6 *N* HCl. After refluxing overnight, evaporation *in vacuo* left the crude amino acid·HCl as a white solid. Passage over Dowex 1 acetate converted the material into the free amino acid which was recrystallized from H_2O–Me_2CO mixtures. The pure acid **8**, mp 269–271° dec, proved to be essentially homogeneous by tlc on silica gel in two solvent systems and was detected as a red spot with ninhydrin: 3:1:1, BuOH–HOAc–H_2O, R_f 0.7; 8:1:1, $CHCl_3$–MeOH–HOAc, R_f 0.15; nmr (D_2O) δ 1.0–2.0 (m, complex, 8, ring CH_2), 3.12 (s, 2, CH_2N), and 6.12 and 6.53 ppm (d's, J = 8.5 Hz, 2, CH=CH); ir (KBr) characteristic absorptions of zwitterionic amino acid. *Anal.* ($C_{10}H_{15}NO_2 \cdot 0.5\ H_2O$) C; H: calcd, 8.42; found, 7.84.

VII. 4-Aminomethylbicyclo[2.2.2]octa-2,5-dione-1-carboxylic Acid (9) (See Schemes I and III). Diethyl 2,5-bis(ethylenedioxo)bicyclo[2.2.2]octane-1,4-dicarboxylate (32) was prepared from the corresponding 2,5-diketo compound as described by Holtz and Stock.[29]

Ethyl hydrogen 2,5-bis(ethylenedioxo)bicyclo[2.2.2]octane-1,4-dica boxylate (33) was prepared in 58% yield from the corresponding diester by saponification of **32** essentially according to the procedure employed by Roberts, *et al.*,[23] and discussed above. Column chromatography on silica gel, using $CHCl_3$ and EtOAc as eluents, enabled clean separation of the desired monoacid **33** from a small amount of the corresponding diacid, produced as a by-product. Recrystallization from C_6H_6, then 95% EtOH, gave pure material, mp 140.5–142°. *Anal.* ($C_{16}H_{22}O_8$) C, H.

Ethyl 4-carbamoyl-2,5-bis(ethylenedioxo)bicyclo[2.2.2]octane-1-carboxylate (34) was prepared from **33** in 64% yield, using the mixed anhydride–anhydrous NH_3 procedure employed by Roberts, *et al.*,[23] for the same compound lacking 2 and 5 substituents. Recrystallization of the solid product from 95% EtOH gave pure material, mp 160.5–162°. *Anal.* ($C_{16}H_{23}NO_7$) C, H, N.

Ethyl 4-cyano-2,5-bis(ethylenedioxo)bicyclo[2.2.2]octane-1-carboxylate (35) was prepared in 84% yield by dehydration of the amide **34** with $POCl_3$. The procedure used was essentially that of Roberts, *et al.*;[23] however, pyridine was employed as a solvent rather than $ClCH_2CH_2Cl$ to prevent acid-catalyzed removal of the labile ketal groups. The solid product **35** was recrystallized from hexane, mp 82.5–84°. *Anal.* ($C_{16}H_{21}NO_6$) C, H, N.

4-Cyano-2,5-bis(ethylenedioxo)bicyclo[2.2.2]octane-1-carboxylic Acid (36).—Saponification of the cyano ester **35** was carried out using previously described conditions[23] and **36** was obtained in about 80% yield, mp 223.5–225°, recrystallized from MeCN. *Anal.* ($C_{14}H_{17}NO_6$) C, H, N.

4-Aminomethyl-2,5-bis(ethylenedioxo)bicyclo[2.2.2]octane-1-carboxylic Acid (10).—To a solution of 2.95 g (0.01 mole) of **36** in 100 ml of EtOH were added 20 ml of H_2O and 500 mg of PtO_2. The mixture was shaken under 2.67 kg/cm^2 of H_2 and after 18 hr the catalyst filtered off and the filtrate evaporated to a white solid. One recrystallization from H_2O–Me_2CO gave 0.97 g (32%) crude material. Tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.6 (ninhydrin, pink, slightly elongated spot); nmr (D_2O) δ 1.5–2.5, sharp peaks at 1.82 and 1.99 (8, bicyclic ring), 3.00 (2, CH_2N), and 3.92 and 4.08 ppm (s, 8, ketal CH_2). Three recrystallizations from H_2O–EtOH gave an analytical sample, mp 238–242°. The ir spectrum (KBr) was as expected for the amino acid bisketal but showed a weak absorption at 1720 cm^{-1} (CO), the same wavelength of the much stronger carbonyl peak in the dione **9**. *Anal.* ($C_{14}H_{21}NO_6$) C, H, N.

4-Aminomethylbicyclo[2.2.2]octa-2,5-dione-1-carboxylic Acid (9).—To 590 mg (2.0 mmoles) of **36** dissolved in 40 ml of EtOH was added 4.0 ml of 1.0 *N* HCl and 100 mg of PtO_2. The mixture was hydrogenated for 2 hr at 2.11 kg/cm^2, filtered, and evaporated, then refluxed for 8 hr with 20 ml of 6 *N* HCl. After evaporation *in vacuo* and reevaporation several times with small portions of H_2O, the crude amino acid·HCl was dissolved in 5 ml of H_2O and passed through a column of Dowex-1 acetate (20 g). Evaporation of the effluent gave the crystalline product, mp >360°. Several recrystallizations from H_2O–Me_2CO gave the pure amino acid for analysis (147 mg), mp >360°. The material appears to darken upon prolonged heating above 100°: tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.40 (ninhydrin, pink); ir (KBr) 1720 (CO), 1610 (COOH) and 1560 cm^{-1} (NH_3^+); equiv wt calcd 221.21, found 223.58; pK_1 3.8, pK_2 8.7 (H_2O). *Anal.* ($C_{10}H_{13}NO_4$) C, H, N.

VIII. 4-(α-Aminoethyl)bicyclo[2.2.2]octane-1-carboxylic Acid

(29) H. D. Holtz and L. M. Stock, *J. Amer. Chem. Soc.*, **86**, 5183 (1964).

(11) (See Scheme IV). **4-Carbomethoxybicyclo[2.2.2]oct-2-ene-1-carbonyl Chloride (37).**—A solution of 5.0 g (0.024 mole) of methyl hydrogen bicyclo[2.2.2]oct-2-ene-1,4-dicarboxylate[26] in 20 ml of $SOCl_2$ was heated to reflux for 3 hr. After removal of the excess $SOCl_2$ *in vacuo*, then reevaporation with several portions of hexane, the resulting material was distilled *in vacuo*, affording 5.3 g (97%) of a colorless liquid, bp 97–98° (7.0 mm).

4-Acetylbicyclo[2.2.2]oct-2-ene-1-carboxylic Acid (38).—A mixture of 0.42 g (0.017 g-atom) of Mg turnings, 2 ml of dry C_6H_6, and 0.1 ml of abs EtOH was heated to reflux until the dissolution of the Mg began. To this was added, over 30 min, a mixture of 2.72 g (0.017 mole) of diethyl malonate, 0.7 ml of abs EtOH, and 3 ml of C_6H_6. After 3 hr at reflux, all the Mg had dissolved. The EtOH was then removed by azeotropic distillation with fresh portions of C_6H_6, the volume brought to about 5 ml with C_6H_6, and a solution of 3.43 g (0.015 mole) of **37** in 5 ml C_6H_6 was added at reflux over a 30-min period. After an additional 3.5 hr at reflux, the viscous mixture was poured into 100 g of ice and 50 ml of 6 *N* HCl. The combined C_6H_6 extracts, after washing (H_2O) and drying ($MgSO_4$), afforded 4.5 g of a colorless liquid, an ir spectrum of which exhibited no COCl absorption. This triester was hydrolyzed directly without purification by refluxing for 16 hr with a mixture of 25 ml of HOAc and 25 ml of concd HCl. Evaporation left 1.83 g (63%) of a white solid, which was chromatographed on silica gel ($CHCl_3$ eluent) to remove a minor slower moving impurity. The chromatographed material was recrystallized from C_6H_6-hexane, giving a white solid: mp 165.5–168°; tlc (sil G) 85:15, C_6H_6–EtOAc, R_f 0.3 (I_2); nmr ($CDCl_3$) δ 1.1–2.2 (m, 8, ring CH_2), 2.23 (s, 3, CH_3CO), 6.56 (s, 2, CH=CH), and about 11 ppm (broad, 1, COOH).

4-(α-Oximinoethyl)bicyclo[2.2.2]oct-2-ene-1-carboxylic Acid (39).—A mixture of 0.52 g (7.5 mmoles) of $H_2NOH \cdot HCl$, 3 ml of abs EtOH, and 3 ml of pyridine was refluxed on a steam bath for 5 min. To this was added 0.49 g (2.5 mmoles) of the keto acid **38**. After 3 hr at reflux, most of the solvent was removed *in vacuo*, then the residue stirred with 30 ml of cold 3 *N* HCl. The resulting crystalline solid was collected, washed with H_2O, and air dried (0.44 g, 85%). The material was recrystallized from 95% EtOH or EtOAc, mp 245–247°.

4-(α-Aminoethyl)bicyclo[2.2.2]octane-1-carboxylic Acid·HCl (11).—To a solution of 270 mg (1.29 mmoles) of **39** in 50 ml of 90% EtOH was added 0.5 ml of 6.0 *N* HCl and 50 mg of PtO_2. The mixture was shaken at 2.11 kg/cm² and 25° for 3 hr. Filtration and evaporation left a white solid which was recrystallized from 95% EtOH–Et_2O, wt 240 mg (80%). The material was dried for analysis at 110° *in vacuo:* mp 313.5–315° dec; tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.57 (ninhydrin, pink); nmr (D_2O) δ 1.13 (d, J = 7 Hz, 3, CH_3), 1.2–2.0 with sharp peaks at 1.55 and 1.66 (12, ring CH_2), and 3.08 ppm (q, J = 7 Hz, 1, CHN); ir (KBr) 1680 (COOH) and 1600 cm⁻¹ (NH_3^+). *Anal.* ($C_{11}H_{19}NO_2 \cdot HCl$) C, H, Cl, N.

IX. 4-Aminobicyclo[2.2.2]octane-1-acetic Acid (13) (See Scheme VI). 4-Acetamidobicyclo[2.2.2]octane-1-carboxylic Acid (40).—To 0.58 g (2.8 mmoles) of 4-aminobicyclo[2.2.2]octane-1-carboxylic acid·HCl (**12**)[23] dissolved in 5 ml of H_2O was added 7.0 ml of 2.0 *N* NaOH. To this was added with stirring (0°) 3 ml of Ac_2O. After 30-min acidification with HCl gave a white solid which was recrystallized from hot H_2O, then dried *in vacuo* over P_2O_5: mp 268.5–270°; 0.40 g (68%); tlc (sil G) 90:25:4, C_6H_6–dioxane–HOAc, R_f 0.30 (H_2SO_4 char); ir (Nujol) 1610 (CONH), 1690 (COOH), and 3300 cm⁻¹ (NH).

4-Acetamidobicyclo[2.2.2]octane-1-carbonyl Chloride (41).—A suspension of 0.31 g (1.47 mmoles) of **40** and 1.0 ml of $SOCl_2$ in 10 ml of pure dry dioxane was refluxed for 2 hr. Evaporation of the clear solution *in vacuo*, reevaporation with several portions of dry C_6H_6, and finally drying *in vacuo* left a yellow oil which was used without further purification.

4-Acetamidobicyclo[2.2.2]octane-1-acetic Acid (42).—The crude acid chloride **41** from 1.70 g (8.1 mmoles) of the corresponding acid **40** was dissolved in 50 ml of dry Et_2O. To this solution at −5° was added dropwise over 30 min an Et_2O solution of CH_2N_2 (25 mmoles). Evaporation of the Et_2O at 25° left a yellow solid; ir ($CHCl_3$) 2120 cm⁻¹ ($COCHN_2$). This material was added in small portions to a stirred mixture of 10 ml of dry $PhCH_2OH$ and 10 ml of distilled collidine at 175° over a 10-min period, then heated an extra 5 min. The resulting brown solution was diluted with 100 ml of EtOAc, then washed (1 *N* HCl, H_2O), and the extracts dried ($MgSO_4$). The filtered solution was distilled *in vacuo* to remove EtOAc and excess $PhCH_2OH$. The resulting brown oil was dissolved in a mixture of 5 ml of abs EtOH and 5 ml of THF, 5.0 ml of 2.0 *N* NaOH was added and the mixture stirred at 25° overnight. After dilution of the reaction mixture with 25 ml of H_2O and extraction with EtOAc, the aq layer was acidified with 6 *N* HCl and the resulting oil extracted into $CHCl_3$ and dried ($MgSO_4$). Evaporation of the extracts and trituration with MeCN afforded a white solid, mp 235–240° (420 mg). Because of the small quantity available the material was hydrolyzed directly without further purification.

4-Aminobicyclo[2.2.2]octane-1-acetic Acid·HCl (13).—A mixture of 420 mg (1.87 mmoles) of **42** and 10 ml of 6 *N* HCl was refluxed for 16 hr, then cooled, and extracted with several portions of EtOAc. Evaporation of the aq layer afforded a pale yellow solid which was triturated with *i*-PrOH, chilled, and the solid collected by filtration. Three recrystallizations from EtOH–Et_2O gave a white solid (105 mg) of constant mp 227–230°: tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.60 (ninhydrin, pink); nmr (D_2O) δ 1.5–1.9 with sharp peaks at 1.67 and 1.80 (12, ring), and 2.12 ppm (s, 2, CH_2COOH). The compound was dried at 110° *in vacuo* for analysis. *Anal.* ($C_{10}H_{17}NO_2 \cdot HCl$) C, H, N.

X. 4-Aminomethylbicyclo[2.2.2]octane-1-acetic Acid·HCl (14) (See Scheme VI). 4-Benzamidomethylbicyclo[2.2.2]octane-1-carbonyl chloride (43) was prepared from the corresponding acid **15b** following the procedure outlined for **41**. The solid product **43**, after drying *in vacuo* over P_2O_5, was used for reaction with CH_2N_2 without further purification.

(4-Benzamidomethylbicyclo[2.2.2]oct-1-yl)diazomethyl ketone (44) was prepared essentially by the procedure described above for the diazo ketone precursor of **42**. The diazo ketone was an oily solid; ir ($CHCl_3$) 2100 cm⁻¹ ($COCHN_2$), free of COCl absorption at 1725 cm⁻¹.

4-Benzamidomethylbicyclo[2.2.2]octane-1-acetic acid (45) was prepared from **44** in the manner described for **42**. The oily product was purified by column chromatography (silica gel, C_6H_6–EtOAc eluent) but could not be obtained crystalline (yield from **15b**, 700 mg, 23%): tlc (Fluor sil G) 90:35:4, C_6H_6–THF–HOAc, R_f 0.66; nmr ($CDCl_3$) δ 1.2–1.8 with sharp peak at 1.52 (12, ring), 2.13 (s, J = 5 Hz, 2, CH_2COOH), 3.21 (d, J = 7 Hz, 2, CH_2N), 6.1–6.4 (broad, 1, NH), and 7.2–8.0 ppm (m, broad, 5, C_6H_5). The material was hydrolyzed directly without further purification to the amino acid **14**.

4-Aminomethylbicyclo[2.2.2]octane-1-acetic Acid·HCl (14).—To 700 mg (2.3 mmoles) of amide acid **45** was added 20 ml of concd HCl and 20 ml of HOAc and the mixture was refluxed overnight. After removal of the solvents *in vacuo*, 50 ml of H_2O was added and the solution extracted thoroughly with Et_2O. Evaporation of the aq layer left a white solid: mp 254–260°; yield 388 mg (72%). Recrystallization from EtOH–Et_2O gave analytically pure material: mp 256.5–260°; tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.50 (ninhydrin, pink); nmr (D_2O) δ 1.50 (s, 8, ring), 2.17 (s, 2, CH_2COOH), 2.76 (s, 2, CH_2N), and 4.62 ppm (s, 4, exchangeable protons). *Anal.* ($C_{11}H_{19}NO_2 \cdot HCl$) C, H, Cl, N.

XI. Amino and Carboxyl Derivatives of 4-Aminomethylbicyclo[2.2.2]octane-1-carboxylic Acid (7). 4-Acetamidomethylbicyclo[2.2.2]octane-1-carboxylic acid (15a) was prepared in 91.6% yield by treatment of an alkaline aq solution of **7**·HCl with Ac_2O at 0°. The solid product was recrystallized from MeCN: mp 223–226°; nmr (CF_3CO_2H) δ 1.3–2.2 with sharp peaks at 1.70 and 1.88 (12, ring), 2.53 (s, 3, CH_3CO), and 3.38 ppm (d, 2, J = 6 Hz, CH_2N); ir (KBr) 1600 (CONH), 1680 (COOH), and 3360 cm⁻¹ (NH). *Anal.* ($C_{12}H_{19}NO_3$) C, H, N.

4-Benzamidomethylbicyclo[2.2.2]octane-1-carboxylic acid (15b) was prepared in 89% yield from **7**·HCl and PhCOCl under well known Schotten–Baumann conditions. The product was recrystallized from C_6H_6–MeCN: mp 179.5–181°; nmr (CF_3CO_2H) δ 0.9–1.8 with sharp peaks at 1.28 and 1.40 (12, ring), 3.03 (d, J = 6 Hz, 2, CH_2N), 7.0–7.5 (m, complex, 5, C_6H_5) and 7.4–8.0 ppm (broad, 1, NH); ir (KBr) 1640 (CONH), 1680 (COOH), and 3380 cm⁻¹ (NH). *Anal.* ($C_{17}H_{21}NO_3$) C, H, N.

4-(*p*-Tolylsulfonamidomethyl)bicyclo[2.2.2]octane-1-carboxylic acid (15c) was prepared from **7**·HCl and TsCl under Schotten–Baumann conditions (yield, 68%). The product was recrystallized from 95% EtOH: mp 263–265°; tlc (Fluor sil G) 90:25:4, C_6H_6—dioxane–HOAc, R_f 0.60; ir (KBr) 1160 and 1330 (SO_2NH), 1690 (COOH), and 3260 cm⁻¹ (NH). *Anal.* ($C_{17}H_{23}NO_4S$) C, H, N, S.

4-(*N*-*p*-Tolylsulfonyl-*N*-methylaminomethyl)bicyclo[2.2.2]octane-1-carboxylic acid (15d) was prepared in 40% yield by treatment of a solution of 0.51 g (1.5 mmoles) of **15c** in 10 ml of 2.0 *N* NaOH with MeI (1.5 g) in a sealed tube at 65° for 3 hr. Evapo-

ration of the MeI and acidification of the aq solution gave the product, which was recrystallized from 95% EtOH: mp 242.5–244°; tlc (as for **15c**), R_f 0.65; ir (KBr) 1160 and 1340 (SO_2NH) and 1680 cm^{-1} (CO_2H). *Anal.* ($C_{18}H_{25}NO_4S$) C, H, N, S.

4-Methylaminomethylbicyclo[2.2.2]octane-1-carboxylic Acid (15e).—A mixture of 0.16 g (0.45 mmole) of **15d**, 10 ml of concd HCl, and 3 ml of HOAc was heated at 100° in a sealed tube for 5 days. After evaporation, the product was dissolved in 3 ml of H_2O and passed through a column of Dowex-1 acetate. After evaporation of the effluent, the crude amino acid was recrystallized from H_2O–Me_2CO: mp 262–264°; yield, 61 mg (69%); tlc (Fluor sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.55 (ninhydrin, pink); ir (KBr) 1390 and 1530 (COO^-) and 1620 and 2400–2600 cm^{-1} ($CH_3N^+H_2$); *Anal.* ($C_{11}H_{19}NO_2$) H; C: calcd, 66.97; found, 69.01.

Methyl 4-Aminomethylbicyclo[2.2.2]octane-1-carboxylate·HCl (15f).—A solution of 0.50 g (2.3 mmoles) of finely powdered **7**·HCl in 50 ml of $SOCl_2$ was heated to reflux for 26 hr. After removal of the $SOCl_2$ *in vacuo*, 50 ml of abs MeOH was added and the solution refluxed for 20 hr. Evaporation and recrystallization of the glassy solid from MeOH–Et_2O afforded fine needles: mp 218.5–220°; yield 310 mg (58%). Material dried at 110° *in vacuo* proved to be a hemihydrate: tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.60 (ninhydrin, pink); ir (KBr) 1720 cm^{-1} ($COOCH_3$); equiv wt calcd, 242.75, found, 237.74; pK 10.0 (H_2O). *Anal.* ($C_{11}H_{19}NO_2 \cdot HCl \cdot 0.5\ H_2O$) C, H, Cl, N.

Benzyl 4-Aminomethylbicyclo[2.2.2]octane-1-carboxylate·HCl (15g).—A solution of 1.0 g (4.6 mmoles) of finely powdered **7**·HCl in 100 ml of $SOCl_2$ was heated at reflux for 32 hr. After removal of the $SOCl_2$ *in vacuo*, 100 ml of $PhCH_2OH$ was added and the solution heated at 105° for 24 hr. Removal of the excess alcohol left a white solid, which crystallized from *i*-PrOH–Et_2O as shiny plates: mp 170–174°; yield 1.15 g (81%); tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.68 (ninhydrin, pink); ir (KBr) 1720 cm^{-1} ($CO_2CH_2C_6H_5$); equiv wt calcd 309.8, found 311.7; pK 9.25 (30% EtOH in H_2O). *Anal.* ($C_{17}H_{23}NO_2 \cdot HCl$) H, Cl, N; C: calcd, 65.90; found, 65.11.

XII. 4-Aminomethylpentacyclo[4.2.0.0.^{2,5}0.^{3,8}0.^{4,7}]octane-1-carboxylic Acid [4-Aminomethylcubane-1-carboxylic Acid (16)] (See Scheme I). Methyl hydrogen cubane-1,4-dicarboxylate (47) was prepared by saponification of dimethyl cubane-1,4-dicarboxylate (**46**)[30] with 1 equiv of NaOH following the procedure of Roberts, *et al.*,[23] for the bicyclo[2.2.2]octane series. The desired product **47** was obtained in 74% yield contaminated with small amounts of the corresponding diacid. Purification was achieved employing a Silicar 7G Analtech Unibar in an ascending fashion using a 90:25:4, C_6H_6–dioxane–HOAc system as the eluent, R_f 0.73 (**47**) and 0.55 (diacid). Product **47** was recrystallized from EtOAc: mp 174.5–176°; nmr (DMSO-d_6) δ 3.70 (s, 3, CO_2CH_3) and 4.22 (s, 6, cube). *Anal.* ($C_{11}H_{10}O_4$) C, H.

Methyl 4-carboxamidocubane-1-carboxylate (48) was obtained in 51.7% yield from **47** by the mixed anhydride–anhydrous NH_3 procedure used by Roberts, *et al.*,[23] in the bicyclo[2.2.2]octane series; using, however, THF as a solvent rather than $CHCl_3$. The product crystallized as lustrous plates from MeOH: mp 238–240° (prior darkening at 228–238°); tlc (sil G) 90:25:4, C_6H_6–dioxane–HOAc, R_f 0.35; nmr (DMSO-d_6) δ 3.63 (s, 3, $COOCH_3$), 4.11 (s, 6, cube), and 6.8–7.3 ppm (broad, 2, $CONH_2$). *Anal.* ($C_{11}H_{11}NO_3$) C, H, N.

Methyl 4-cyanocubane-1-carboxylate (49) was prepared in 79% yield from the amide ester **48** by dehydration with $POCl_3$ in $(ClCH_2)_2$ according to the method of Roberts, *et al.*[23] The cyano-ester was recrystallized from abs EtOH: mp 145.5–147°; tlc (sil G) 9:1, C_6H_6–MeOH, R_f 0.65 (H_2SO_4 char); nmr ($CDCl_3$) δ 3.70 (s, 3, $COOCH_3$) and 4.32 ppm (s, 6, cube); ir (KBr) 2220 (CN) and 1720 cm^{-1} ($COOCH_3$). *Anal.* ($C_{11}H_9NO_2$) C, H.

4-Cyanocubane-1-carboxylic acid (50) was obtained in 81% yield by saponification of **49** using the conditions described by Roberts, *et al.*,[23] in the bicyclo[2.2.2]octane series. The product (**50**) was purified by column chromatography (silica gel with $CHCl_3$–EtOAc eluents): mp 196–202°; ir (KBr) 2220 (CN) and 1690 cm^{-1} (COOH).

4-Aminomethylcubane-1-carboxylic Acid (16).—To 156 mg (0.90 mmole) of **50** dissolved in 25 ml of MeOH was added 10 ml of distilled H_2O, 0.50 ml of 6.0 N HCl, and 100 mg of PtO_2. The mixture was hydrogenated at 2.46 kg/cm² for 70 min. After filtration to remove the catalyst, evaporation left the crude amino acid·HCl: nmr (D_2O) δ 3.32 (s, 2, CH_2N) and an A_3B_3 pattern centered at 4.11 ppm (6, cube). For purification, the hydrochloride was converted into the free amino acid by passage of an aq solution over a column of Dowex-1 acetate and recrystallization (needles) from H_2O–Me_2CO. The pure material (**16**) decomposed without melting at 245–255° when placed in a preheated oil bath at 180°: tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.60 (ninhydrin, yellow); ir (KBr) 2130, 1640, 1625, 1560, 1530, 1500, 1460, 1400 (broad), 1310, 1240, 1200, 1170, 1160, and 760 cm^{-1}. *Anal.* ($C_{10}H_{11}NO_2$) C, H, N.

XIII. 6-Aminomethylspiro[3.3]heptane-2-carboxylic Acid (17) (See Scheme I). Methyl hydrogen spiro[3.3]heptane-2,6-dicarboxylate (51) was prepared in 48.6% yield from dimethyl spiro[3.3]heptane-2,6-dicarboxylate[31] and 1 equiv of NaOH, using the procedure of Roberts, *et al.*[23] The mono acid **51** was chromatographed on silica gel using $CHCl_3$ as an eluent to remove a small amount of the corresponding diacid. The product was recrystallized from hexane: mp 53–55°; tlc (sil G) 90:25:4, C_6H_6–dioxane–HOAc, R_f 0.71. *Anal.* ($C_{10}H_{14}O_4$) H; C: calcd, 60.59; found, 61.01.

Methyl 6-carbamoylspiro[3.3]heptane-2-carboxylate (52) was prepared from **51** in 64.5% yield by the usual mixed anhydride anhydrous NH_3 procedure.[23] The product crystallized as shiny plates from C_6H_6: mp 148.5–150°; tlc (sil G) 90:25:4, C_6H_6–dioxane–HOAc, R_f 0.50. *Anal.* ($C_{10}H_{15}NO_3$) C, H, N.

Methyl 6-cyanospiro[3.3]heptane-2-carboxylate (53) was obtained from **52** as an oil in about 60% yield by the usual $POCl_3$ dehydration procedure:[23] tlc (sil G) $CHCl_3$, R_f 0.30; ir (neat) 2210 cm^{-1} (CN).

6-Cyanospiro[3.3]heptane-2-carboxylic acid (54) was prepared from **53** and NaOH according to the usual procedure.[23] The material was obtained in 43% yield from **52**, mp 98–101°; tlc (sil G) 90:25:4, C_6H_6–dioxane–HOAc, R_f 0.78.

6-Aminomethylspiro[3.3]heptane-2-carboxylic Acid (17).—To 250 mg (1.51 mmoles) of **54** dissolved in 20 ml of abs EtOH were added 0.50 ml of 6.0 N HCl and 100 mg of PtO_2. The mixture was hydrogenated at 2.11 kg/cm² and room temperature for 1 hr, then left to evaporate overnight. Subsequent studies indicated that the desired amino acid·HCl was contaminated with a small quantity of the amino ester produced during hydrogenation. Hydrolysis for 6 hr with 10 ml of 3 N HCl produced the pure amino acid·HCl (41%), mp 154–158°. Dissolution in H_2O and passage over a column of Dowex-1 acetate afforded the free amino acid: 68 mg; dec 255–260° (H_2O–Me_2CO); tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.70 (ninhydrin, pink); equiv wt calcd, 169.22, found, 168.96; pK_1 4.4, pK_2 10.2 (H_2O). *Anal.* ($C_9H_{15}NO_2$) H, N; C: calcd, 63.88; found, 63.39.

XIV. 6-Aminospiro[3.3]heptane-2-carboxylic acid (18) (see Scheme V) was prepared in about 30% yield from the amide ester **52** by treatment with Br_2 and NaOMe, followed by acid hydrolysis of the resulting urethane to the amino acid **18**, according to the procedure used by Roberts, *et al.*,[23] for the preparation of **12**. The resulting hydrochloride was converted into the free amino acid by passage over Dowex-1 acetate and the product recrystallized from H_2O–Me_2CO. An air-dried hydrate, mp 233–236° afforded the pure amino acid after drying at 100° *in vacuo*: mp 218–220°; tlc (sil G) 3:1:1, BuOH–HOAc–H_2O, R_f 0.45 (ninhydrin, red); equiv wt calcd, 155.19, found, 145–150; pK_1 4.3, pK_2 9.9 (H_2O). *Anal.* ($C_8H_{13}NO_2$) H, N; C: calcd, 61.91; found, 62.98.

Biological Assay Methods.—Antifibrinolytic activity *in vitro* was assessed by two statistically reliable methods. These are described briefly; the detailed procedures have been published.[22]

Method A. Inhibition of Clot Lysis Effected by Streptokinase-Activated Human Plasminogen.—Human fibrin clots were prepared by the addition of thrombin to a standard amount of human fibrinogen, human plasminogen, and streptokinase. The time for complete lysis was measured at 37° in the presence and absence of inhibitors. The concentration of the inhibitor which would increase the geometric mean lysis time by 50% was estimated. A concentration of 0.05 and 0.2 mg/ml of ε-aminocaproic acid was used as a standard inhibitor preparation. Comparison of the test compound and the standard EACA preparation permitted the evaluation of the test substance potency relative to the standard.

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Method B. Inhibition of Chemically Induced Lysis of a Preformed Plasma Clot.—The clots were prepared from human plasma containing ^{125}I-labeled human fibrinogen by the addition of $CaCl_2$ and bovine thrombin. After thorough washing to remove loosely bound radioactivity, fibrinolysis was initiated by the addition of *o*-thymotic acid (6-methyl-3-isopropylsalicylic acid) to the suspending medium and was measured by the release of radioactivity into the medium. Lysis was prevented if an antifibrinolytic compound was present in the ambient solution. Inhibition of the release of radioactivity from the plasma clot into the ambient solution is directly proportional to inhibition of lysis. From these data, the relative potency of the antifibrinolytic compound was calculated.

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Potential Anticancer Agents. III. Schiff Bases from Benzaldehyde Nitrogen Mustards and Aminophenylthiazoles

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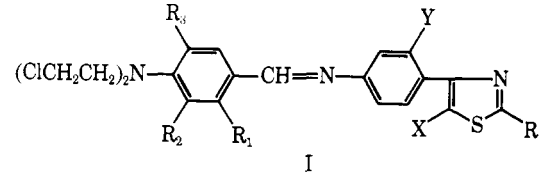
Several Schiff bases from different benzaldehyde nitrogen mustards and amines such as 4-(*p*-aminophenyl)-2,5-disubstituted-thiazoles and 4-[(4′-amino-2′-chloro)phenyl]-2-substituted-thiazoles have been synthesized and screened for antitumor activity. Many of the compounds displayed significant activity against L 1210 lymphoid leukemia, Walker 256 (intramuscular), and Dunning leukemia (solid).

The nonspecific cytotoxic effect of the N mustards has limited their use in the chemotherapy of cancer. The concept of "latent activity" whereby the drug is so designed as to be inactive *per se* but gets modified into an active form by processes taking place in the target cells has been very fruitful in the search for better antitumor agents. Ross and coworkers[2] synthesized azomustards while Popp[3] studied several Schiff bases of benzaldehyde N mustards and found them active enough in an experimental tumor system to merit clinical trials. Following this lead we have reported[4] in an earlier communication the synthesis and study of Schiff bases from substituted benzaldehyde N mustards and various arylamines. A number of compounds from this series displayed significant activity against Dunning leukemia (solid), lymphoid leukemia (L-1210), and Walker carcinosarcoma 256 (intramuscular). The substituent on the benzaldehyde N mustard greatly influenced the activity and specificity and the presence of a halogen in the *meta* position of the arylamine induced activity of a high order. Another significant observation in our earlier work was that among arylamines, the 4-(*p*-aminophenyl)thiazoles afforded more active Schiff bases. In view of these findings the work has now been extended and Schiff bases of structure I from substituted benzaldehyde N mustards and various 4-(*p*-aminophenyl)thiazoles have been prepared and screened to study the role of different substituents in the molecule.



I

X = Me and H
Y = Cl and H

R = M, $PhCH_2$, $PhOCH_2$ *o*- or *p*-toluoxymethyl, Ph, *p*-MePh, *p*-MeOPh, and *p*-ClPh
R_1 = H, OMe, Me, and F
R_2 = H, OMe and OEt
R_3 = H and OMe

Chemistry.—The general method adopted for the preparation of Schiff bases, *viz.*, heating a mixture of the amine and aldehyde in EtOH, though successful in certain cases was not particularly useful when the aldehyde was a liquid. In such cases the resulting compounds were invariably viscous oils which could not be induced to crystallize. In a few cases the modified method recommended by Tipson and Clapp[5] involving heating under reflux a mixture of amine and aldehyde in PhMe containing a few drops of piperidine was tried. The procedure, though successful when carried out with smaller amounts did not give pure products in larger quantities. The most suitable method found in the present work was the heating of pure amine hydrochloride with mustard aldehyde in EtOH.[6] In a short time the highly colored hydrochloride of the Schiff base separated out and the product was invariably found to be analytically pure with yields varying between 60 and 70% (Table I).

The required aldehyde mustards were prepared by hydroxyethylation of various anilines with ethylene oxide[7] and then treating the products with $POCl_3$ in DMF.[8] The requisite 4-(*p*-aminophenyl)thiazoles

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