water was added in the same sequence at 30-sec intervals to stop the reaction with subsequent formation of a fine precipitate. This precipitate was separated by centrifugation at 2500 rpm for 5 min or was allowed to settle overnight in a refrigerator (~5°) with retention of 90–95% of the original absorbance. The optical density of each sample was then read (666 nm) against reaction mixtures from each time period containing all components except microsomes (i.e., substrate blanks). A Perkin-Elmer Coleman 101 spectrophotometer was used for the readings.

Calculations. Aldehydes present in the microsomes (0.1–0.6 μg/ml) were determined simultaneously at each time interval using incubation mixtures containing all components but substrate. The quantity of this native aldehyde was subtracted from that value obtained for the reaction mixtures at the same time period. Apparent K_m and V_{max} values were determined from the amount of acetaldehyde formed at several substrate concentrations (0.125, 0.250, 0.500, 1.00, and 1.50 mM) during a 7.5-min incubation period. The data were fitted to the equation $S/v = S/V_m + K_m/V_m$ by linear regression analysis of S/v on S. Initial velocity determinations were made using the amount of acetaldehyde evolved at 0, 2.5, 5, and 7.5 min from reactions 1.00 mM in substrate. A least-squares fit of the data to a straight line was used to obtain the slopes and thus the k_H/k_D values. All velocities of deuterated substrates were corrected for less than 100% deuterium incorporation (lidocaine-d_4, 89%, and lidocaine-d_6, 94%). The Student's t test was used for statistical analysis.

Mass Spectral Determination of Trapped Acetaldehyde. A microsomal experiment was performed with lidocaine-d_6 (**3**) as previously described, and the acetaldehyde released during a 7.5-min incubation was allowed to react with 100 μl of the 3-methyl-2-benzothiazolone hydrazone reagent (0.5%) utilizing the same procedure described for the colorimetric determinations except the ferric chloride step was omitted to avoid further oxidation of the intermediate azine. The intermediate azine was extracted into purified ether at pH 6.5–7.0, the extract was filtered through anhydrous magnesium sulfate, and the filtrate was rotary evaporated at ambient temperature to yield a yellow residue which was subjected to chemical ionization mass spectrometry with isobutane as reagent gas at 140°.

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Hypo-β-lipoproteinemic Agents. 1. Bicyclo[2.2.2]octyloxyaniline and Its Derivatives

Charles E. Day, Paul E. Schurr, D. Edward Emmert, Ruth E. TenBrink, and Daniel Lednicer*

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan 49001. *Received June 30, 1975*

A new assay for agents which normalize β-lipoproteins in cholesterol–cholic acid fed rats is described. Both lowering of serum cholesterol and of serum heparin precipitable lipoproteins (HPL) were measured at the end of the treatment period. Compounds which shifted the ratio of the decrease in favor of HPL are considered hypo-β-lipoproteinemic. *p*-(1-Bicyclo[2.2.2]octyloxy)aniline and several of its derivatives proved active in this assay. The synthesis of these compounds is described.

All serum lipids are bound to proteins. These lipoproteins are broadly divided into the four classes: chylomicrons, very low density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). During electrophoresis VLDL, LDL, and HDL migrate with pre-β, β, and α mobilities, respectively.[1] In the normal adult human approximately two-thirds of the total serum cholesterol is associated with the cholesterol rich LDL. Total serum cholesterol level is usually a reflection of serum LDL concentration. Since both chylomicrons and VLDL are triglyceride rich, elevated serum triglyceride levels simply reflect an increase in either one or both of these entities.[1]

Elevations of serum lipid levels have been grouped into five basic types as classified by lipoprotein patterns. The most common hyperlipoproteinemias are types II and IV, an abnormal increase in LDL and VLDL, respectively.[1] In both these hyperlipoproteinemias the incidence of prema-

ture cardiovascular disease is significantly increased. Victims of the most severe form of type II (familial homozygous hyper-β-lipoproteinemia) frequently die from coronary heart disease at an early age (as early as 18 months) and seldom live more than 30 years.[1] By contrast, there appears to be no increased incidence of cardiovascular disease in persons with hyperchylomicronemia.[1] Elevations of HDL are quite rare.

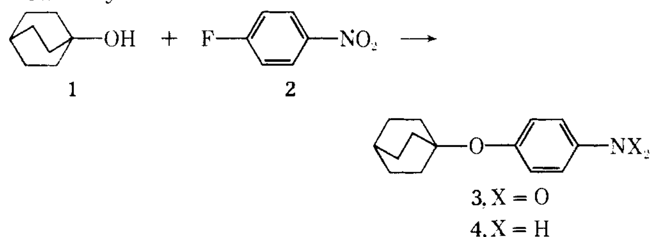
In the few cases that have been studied there is no indication of premature vascular disease.[2] Thus LDL and VLDL, but not chylomicrons and HDL, may be termed atherogenic lipoproteins.

Instead of agents to reduce serum cholesterol, it would possibly be more advantageous to develop agents that specifically reduce either one or both of the atherogenic lipoproteins. An increase in HDL might be desirable also. Assuming the desirability of such agents, the problem arises as to their method of discovery. The rodent models used for the random screening of hypolipidemic agents are not satisfactory since their serum lipoproteins consist predominantly of HDL. In an effort to find a suitable animal model we examined the lipoprotein electrophoretic patterns of numerous animal species.[3] However, only pigs, opossums, and snakes possessed a moderately high concentration of LDL. Because of the unsuitability of these animals for screening purposes, we focused our attention on dietary induced hyperlipoproteinemia in rodents.

Cholesterol-cholic acid fed weanling rats develop a fairly severe hypercholesterolemia. Most of the cholesterol in hypercholesterolemic weanling rat serum is in the VLDL density range of $d < 1.006$ g/ml. The lipoprotein itself has many of the properties both of VLDL and LDL and has been designated β-VLDL or cholesterol ester rich VLDL.[4] Although not ideal for a model of either type II or IV hyperlipoproteinemia in humans, such lipoproteins are markedly atherogenic in rabbits, pigeons, and other species.

The atherogenic lipoproteins (VLDL, LDL, and β-VLDL) are precipitated from diluted rat serum by heparin in the presence of calcium ions. As determined by preparative ultracentrifugation at 1.040 g/ml, only lipoproteins with $d < 1.040$ g/ml are precipitated by heparin. The precipitate that develops can be measured turbidimetrically quite conveniently. HDL is not precipitated by heparin. In screening for hypolipoproteinemic agents we routinely measured serum cholesterol levels and heparin precipitating lipoproteins (HPL) by turbidimetry. Theoretically, a decrease in the ratio of HPL–cholesterol should indicate a selective decrease in the atherogenic HPL and/or an increase in the HDL. Agents that significantly reduce both the HPL and HPL–cholesterol ratio are designated hypo-β-lipoproteinemic agents.

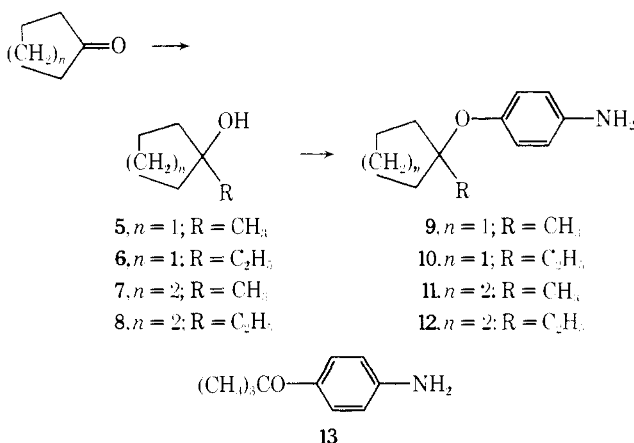
The Triton assay[8] has in the past proven fairly reliable as a means for the detection of hypocholesteremic agents. In order to compare the effect on serum cholesterol in the current assay system, all compounds were screened in the Triton assay as well. Random screening identified bicyclo[2.2.2]oct-1-yloxyaniline (4) as an agent active not only in the Triton screen but one which effected a significant decrease in the HPL–cholesterol ratio as determined by the new assay.



Chemistry. Nucleophilic aromatic substitution of the anion obtained from treatment of bicyclo[2.2.2]octan-1-ol[9] with sodium hydride on p-fluoronitrobenzene gave the ether **3** in moderate yield. Reduction of the nitro group by means of catalytic hydrogenation led to the desired substituted aniline **4**.

In order to ascertain whether the bicyclo[2.2.2]oct-1-yl moiety (accessible by a rather lengthy synthesis)[9] was in fact necessary for biological activity, we prepared ethers of aniline substituted with groups of comparable lipophilic and steric characteristics. Thus, alkylation of p-fluoronitrobenzene with the tertiary cyclic alcohols **6–9** (obtained by reaction of the corresponding cycloalkanone with methyl and ethyl Grignard reagents) afforded the corresponding nitrophenol ethers. These oily products were separated by chromatography and reduced to the anilines **10–12** without further characterization. p-*tert*-Butoxynitrobenzene was reduced to the corresponding known aniline **13**[10] as well (Scheme I).

Scheme I



We next turned our attention to modification of the amine function of **4** (Scheme II). Thus alkylation with 1,4-

Scheme II

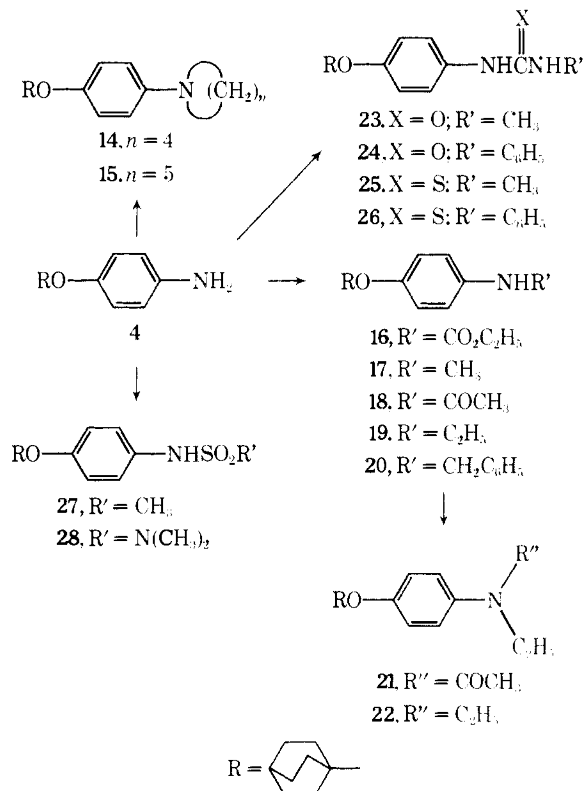
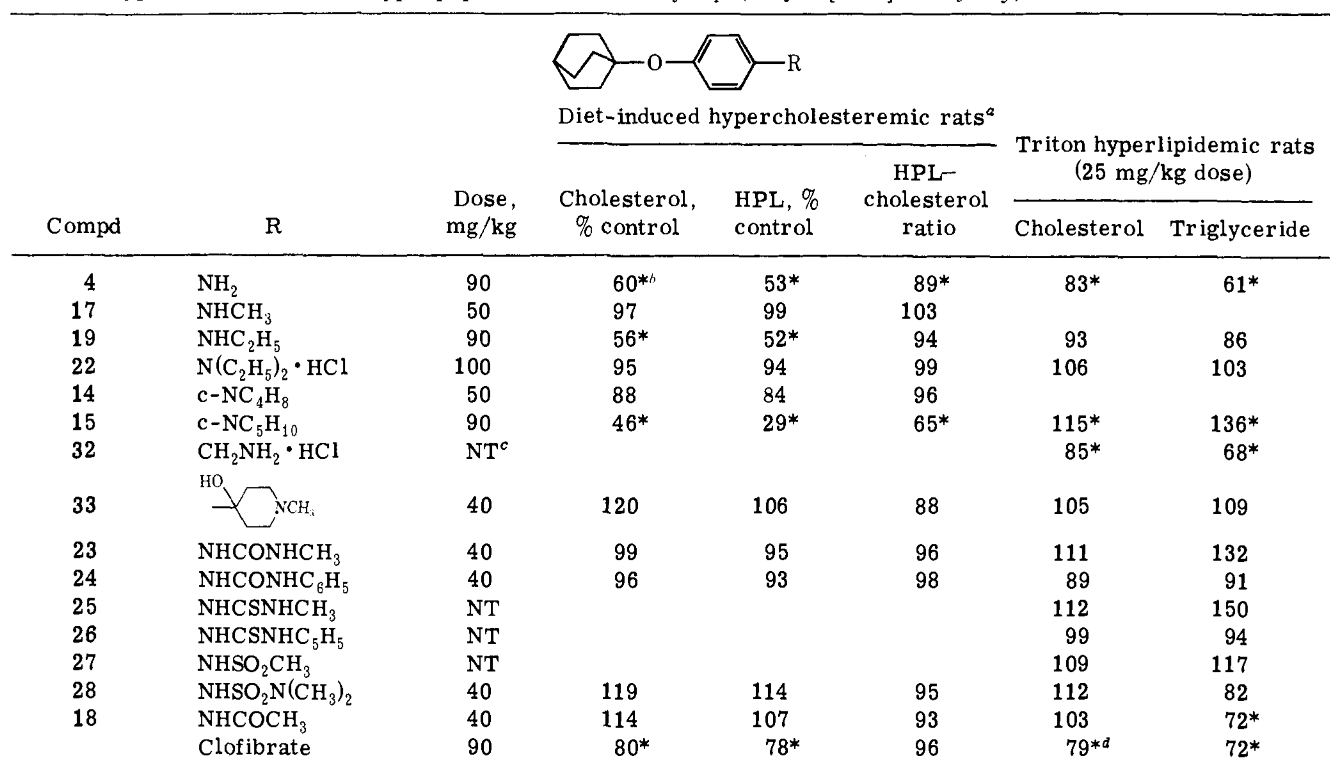


Table I. Hypocholesteremic and Hypolipoproteinemic Activity of *p*-(Bicyclo[2.2.2]oct-1-yloxy)anilines



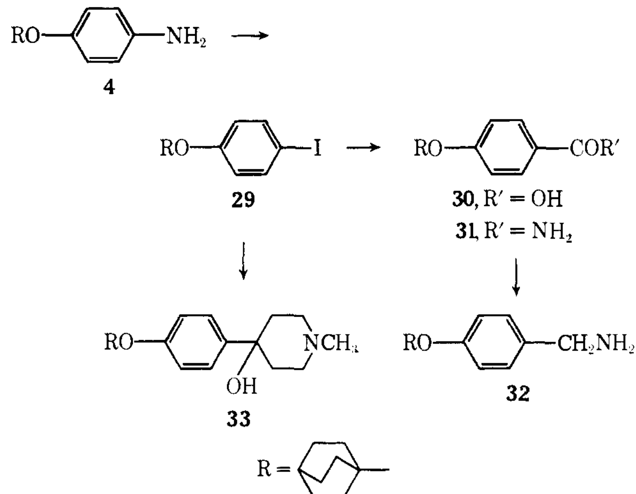
Compd	R	Dose, mg/kg	Diet-induced hypercholesteremic rats[a]: Cholesterol, % control	HPL, % control	HPL–cholesterol ratio	Triton hyperlipidemic rats (25 mg/kg dose): Cholesterol	Triglyceride
4	NH_2	90	60*[b]	53*	89*	83*	61*
17	$NHCH_3$	50	97	99	103		
19	NHC_2H_5	90	56*	52*	94	93	86
22	$N(C_2H_5)_2 \cdot HCl$	100	95	94	99	106	103
14	c-NC_4H_8	50	88	84	96		
15	c-NC_5H_{10}	90	46*	29*	65*	115*	136*
32	$CH_2NH_2 \cdot HCl$	NT[c]				85*	68*
33	HO, NCH_3 (4-piperidyl ring)	40	120	106	88	105	109
23	$NHCONHCH_3$	40	99	95	96	111	132
24	$NHCONHC_6H_5$	40	96	93	98	89	91
25	$NHCSNHCH_3$	NT				112	150
26	$NHCSNHC_5H_5$	NT				99	94
27	$NHSO_2CH_3$	NT				109	117
28	$NHSO_2N(CH_3)_2$	40	119	114	95	112	82
18	$NHCOCH_3$	40	114	107	93	103	72*
	Clofibrate	90	80*	78*	96	79*[d]	72*

[a]Control values for serum cholesterol and HPL were 608 (mg/dl) and 820 ($A_{680} \times 10^3$), respectively. Overall percent pooled standard deviation was 25 and 23% for cholesterol and HPL. [b]* denotes a response significantly different ($p = <0.05$) from control means. [c]Not tested. [d]100 mg/kg.

dibromobutane afforded the pyrrolidine **14**, while the analogous reaction with 1,5-diiodopentane gave the piperidine **15**. The monoalkylated compounds **17** and **19** were obtained by reduction of the corresponding monoacyl derivatives. The benzylated amine **20** was prepared by reduction of the Schiff base obtained from **4** and benzaldehyde. The diethyl analog **22** was obtained by a second acylation–reduction scheme starting with **19**. Reaction on the aniline **4** with the appropriate isocyanates and isothiocyanates afforded the ureas and thioureas **23–26**. Finally reaction of the aniline with respectively methanesulfonyl chloride and *N,N*-dimethylsulfamyl chloride gave the sulfonamides **27** and **28** (Table I).

Initial attempts to modify the aromatic amine function via its diazonium salt by classical methods led to fragmentation of that intermediate; attempts to form the salt afforded bicyclo[2.2.2]octan-1-ol and hydroquinone as the only isolable products. Resort to a recently developed aprotic counterpart of the Sandmeyer reaction[11] did, however, afford a modest yield of the iodo derivative **29**. This last was converted to the corresponding acid by carbonation of the lithium derivative obtained by lithium–halogen interchange. Conversion of the acid to the amide by standard means followed by reduction with lithium aluminum hydride gave the amine **32** homologous with the lead compound. Reaction of the lithium reagent from **29** with *N*-methyl-4-piperidone gave the derivative **33**, containing a basic nitrogen remote from the aromatic ring (Scheme III).

Scheme III

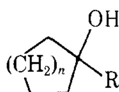


Pharmacology. Weanling albino Upj:TUC(SD)spf rats were housed in groups of five animals and allowed free access to experimental diet and water for 3 days before being distributed by weight into experimental groups of four to six animals. The semisynthetic diet of Phillips and Berg[5] was used, with coconut oil substituted for corn oil and 18% casein and 0.2% methionine as the protein source. Also, 1.5% cholesterol and 0.5% cholic acid were added to the diet at the expense of dextrin. Drugs were dispersed in vehicle (0.25% aqueous methylcellulose) and administered by gastric intubation. Controls received similar volumes of vehicle. After the fourth dose of drug, animals were weighed, fasted 17 hr, and anesthetized with sodium cyclopal. Blood was collected from severed throats.

Individual serum samples were analyzed for total cholesterol[6] and HPL. HPL were determined by the method of Schurr and Day.[7] Both the automated cholesterol and HPL assays were computer interfaced to eliminate manual data reduction. Programs to determine statistically significant differences ($p \leq 0.05$) from control means as determined by Students' test using pooled error variance were included in the computer interface.

Compounds were assayed for their hypocholesteremic effect in the Triton assay in groups of ten animals by the

Table II. 1-Alkylcycloalkyl-1-ols



n	R	% yield	Bp, °C (mmHg)
1	CH_3	44	57–63 (33)
1	C_2H_5	59	77–80 (46)
2	CH_3	74	64–65 (18)
2	C_2H_5	73	80–81 (25)

method described earlier.[8] Serum samples were analyzed for cholesterol and triglycerides; data were analyzed by the same statistical methods as above.

The effects of the *p*-(bicyclo[2.2.2]oct-1-yloxy)aniline derivatives on serum lipids in the two model systems for hyperlipidemias as well as on HPL and the HPL–cholesterol ratio are recorded in Table I. Those analogs lacking the bicyclic ether (**9–14**) were uniformly without effect on any of the parameters assayed at the top screening dose for each test (90 mg/kg for hypo-β-lipoproteinemic assay and 25 mg/kg for the Triton assay).

Discussion

Examination of the screening data shows some degree of correspondence between the two test systems. Thus, both compounds which show a shift in the HPL–cholesterol ratio (**4**, **15**) show a statistically significant response in the Triton assay. It should, however, be noted that the agent which causes the largest shift in the HPL–cholesterol ratio is a hyperlipidemic agent in the Triton assay. It is of note, too, that while **19** and clofibrate lower both cholesterol and HPL in the dietary induced hypercholesteremic rat, these compounds do so without affecting the ratio, the implication being that no shift of cholesterol has occurred away from the atherogenic lipoproteins. Activity in this series seems to have some fairly rigid structural requirements. The apparent requirement for the bridged polycyclic ether is particularly interesting. The data further indicate that the presence of a basic group on the aromatic ring is required for activity. Thus, simple acetylation (**18**) of the lead compound causes loss of activity.

Experimental Section[12]

1-Alkylcycloalkyl-1-ols. To an ice-cooled solution of 0.40 mol of methyl- or ethylmagnesium bromide in ether there was added a solution of 0.30 mol of cyclopentanone or cyclohexanone in 250 ml of ether. Following 2 hr of standing at room temperature the mixture was cooled in ice and treated with 230 ml of saturated aqueous ammonium chloride. The organic layer was separated, washed with water and brine, and taken to dryness. The residual oil was distilled at reduced pressure to afford the tertiary alcohols. The identity of the products was established by ir and NMR spectra (Table II).

***p*-(Bicyclo[2.2.2]oct-1-yloxy)nitrobenzene (3).** A mixture of 10 g of bicyclo[2.2.2]octan-1-ol and 3.37 g of 56% sodium hydride in mineral oil in 163 ml each of benzene and DMF was heated at gentle reflux for 45 min. The mixture was cooled to room temperature and 12.4 g of *p*-fluoronitrobenzene was added. The mixture was again brought to reflux. Following 15 hr of heating the dark mixture was allowed to cool and washed thoroughly with water and then brine. The residue which remained when the solution was taken to dryness was chromatographed on 1 l. of silica gel. Elution with 20% benzene in Skellysolve B afforded first recovered fluoronitrobenzene; elution with benzene gave crude product.

The product was recrystallized from ether–petroleum ether to afford 7.50 g (38%) of yellowish crystals, mp 91.5–94°. This analytical sample melted at 92–94.5°. Anal. ($C_{14}H_{17}NO_3$) C, H.

Ethers of *p*-Aminophenol. In a typical experiment 2.10 g of sodium hydride (56% in mineral oil) was added to a solution of 0.05 mol of the alcohol in 60 ml of DMF and 120 ml of benzene. The mixture was heated at reflux for 30 min and then cooled to room temperature. *p*-Fluoronitrobenzene (7.05 g) was then added and the mixture heated at reflux for 6 hr. The solution was then worked up in the usual way. The residual oil was chromatographed on 1 l. of silica gel (elution with 2 l. each of 20% benzene–Skellysolve B, 40% benzene–Skellysolve B and benzene). The fractions which were similar by TLC were combined to give the nitro ether as a yellow oil.

A mixture of the oil and 1.0 g of 10% palladium on charcoal in 200 ml of ethanol was shaken under hydrogen until the uptake of gas (3 equiv) stopped. The catalyst was removed by filtration and the filtrate taken to dryness. The residue was purified by either distillation or crystallization (Table III).

***N*-[(4′-Bicyclo[2.2.2]oct-1-yloxy)phenyl]pyrrolidine (14).** A mixture of 2.17 g (10 mmol) of the primary amine, 2.8 g of K_2CO_3, and 2.18 g (1.2 ml) of $Br(CH_2)_4Br$ in 25 ml of EtOH was heated at reflux for 20 hr. The mixture was diluted with H_2O and this extracted with Et_2O. The organic layer was washed with H_2O and brine and taken to dryness. The residual solid was recrystallized several times from MeOH to give 1.63 g (60%) of product, mp 99–100°. Anal. ($C_{18}H_{25}NO$) C, H, N.

***N*-[(4′-Bicyclo[2.2.2]oct-1-yloxy)phenyl]piperidine (15).** Proceeding exactly as above 10 mmol of the primary amine was alkylated by means of 3.25 g of 1,5-diiodopentane. The product was isolated as above and then chromatographed on 250 ml of silica gel (elution with NH_3 saturated C_6H_6). The crystalline fractions were recrystallized from petroleum ether to give 0.85 g (30%) of product, mp 66–68°. Anal. ($C_{19}H_{27}NO$) C, H.

***N*-Carbethoxy-4-(bicyclo[2.2.2]oct-1-yloxy)aniline (16).** To an ice-cooled solution of 5.0 g (23 mmol) of the primary amine in 25 ml of pyridine there was added dropwise 4.4 ml of $C_2H_5O_2CCl$. Following 5 hr of standing in the cold the mixture was diluted to 200 ml with ice–H_2O. The precipitated solid was recrystallized from MeOH to afford 5.82 g (87.5%) of product, mp 126.5–127.5°. Anal. ($C_{17}H_{23}NO_3$) C, H, N.

4′-(Bicyclo[2.2.2]oct-1-yloxy)acetanilide (18). A solution of 3.0 g (14 mmol) of the amine and 1.5 ml of acetic anhydride in 50 ml of THF was allowed to stand at room temperature for 5 hr. The bulk of the solvent was removed in vacuo and the residue suspended in water. The solid was collected on a filter and recrystallized from aqueous methanol. There was obtained 3.15 g (87%) of amide, mp 183–185°. Anal. ($C_{16}H_{21}NO_2$) C, H.

4′-(Bicyclo[2.2.2]oct-1-yloxy)-*N*-ethylacetanilide (21). Proceeding exactly as above the free base from 3.0 g (10 mmol) of the hydrochloride of **19** was acetylated with acetic anhydride. The product was recrystallized from Skellysolve B to afford 2.35 g (82%) of product, mp 99–101°. Anal. ($C_{18}H_{25}NO_2$) C, H.

Benzal-*p*-(bicyclo[2.2.2]oct-1-yloxy)aniline. A solution of 9.70 g (0.045 mol) of the primary amine and 4.70 g (0.044 mol) of benzaldehyde in 250 ml benzene was heated at reflux under a Dean–Stark trap for 8 hr. The solution was then taken to dryness. The residue (14.42 g, mp 145–156°) was reduced with lithium aluminum hydride (see below) without further characterization.

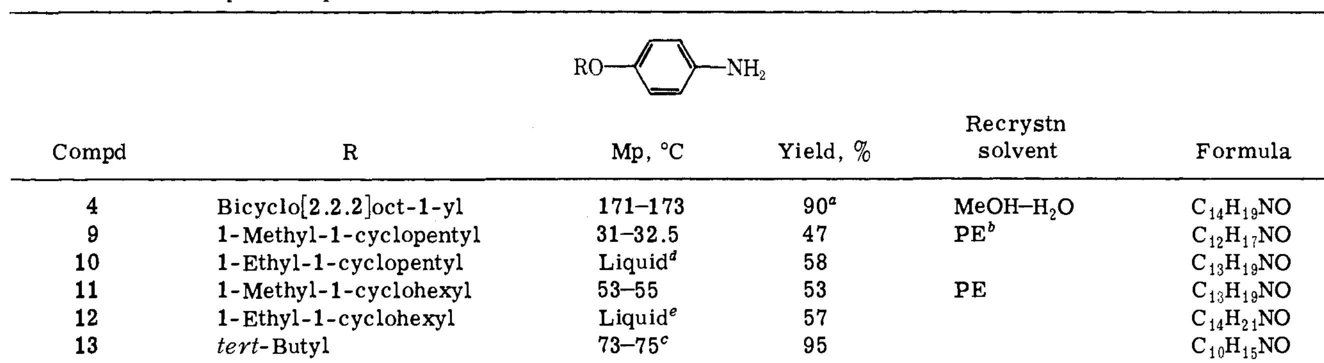
***N*-Alkyl-*p*-(bicyclo[2.2.2]oct-1-yloxy)anilines.** A solution of 0.045 mol of the substrate (amide, carbamate, or Schiff base) in 250 ml of THF was added to a well-stirred suspension of 7 g (0.18 mol) of lithium aluminum hydride in 75 ml of THF. Following 5 hr of heating at reflux the mixture was cooled in ice and treated in turn with 7 ml of water, 7 ml of 15% sodium hydroxide, and 21 ml of water. The inorganic gel was collected on a filter and the filtrate taken to dryness. The residue if solid was recrystallized. If not, the compound was converted to its hydrochloride salt and recrystallized as such. In the case of the tertiary amine, the product was first chromatographed on silica gel (elution with 1:1 benzene–chloroform saturated with ammonia) and then recrystallized as its hydrochloride (Table IV).

Ureas and Thioureas. In a typical example a solution of 1 g (4.6 mmol) of the primary amine in 20 ml of THF was treated with 6.4 mmol of the appropriate heterocumulene. Following 18 hr of stirring at room temperature, the mixture was diluted with water. The precipitated solid was then recrystallized (Table V).

4′-(Bicyclo[2.2.2]oct-1-yloxy)methanesulfonanilide (27). Methanesulfonyl chloride (1 g) was added dropwise to a solution of 1.50 g (6.9 mmol) of the aniline in 15 ml of pyridine. The red solution was allowed to stand at room temperature for 4.5 hr and then poured into water. The precipitate was collected on a filter and recrystallized twice from aqueous methanol. There was obtained 1.70 g (84%) of solid, mp 134–136°. Anal. ($C_{15}H_{21}NO_3S$) C, H, S.

***N*-[*p*-(Bicyclo[2.2.2]oct-1-yloxy)phenyl]-*N′*,*N′*-dimethyl-

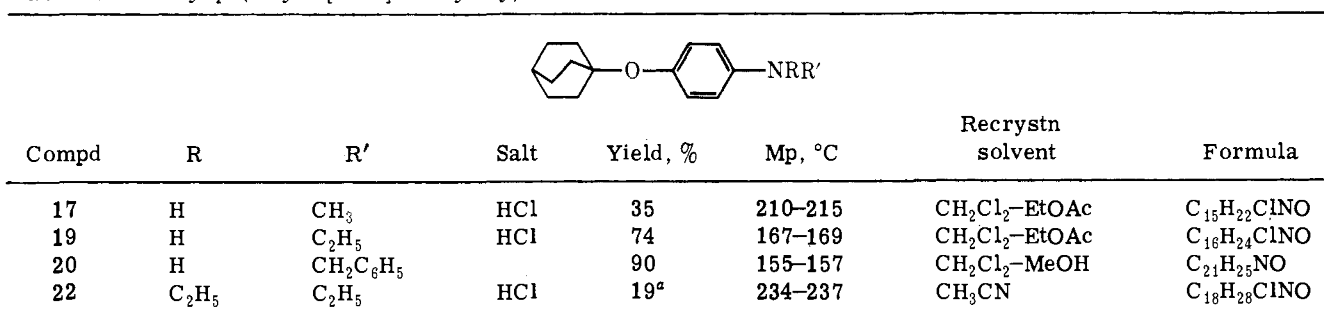
Table III. Ethers of *p*-Aminophenol



Compd	R	Mp, °C	Yield, %	Recrystn solvent	Formula
4	Bicyclo[2.2.2]oct-1-yl	171–173	90[a]	MeOH–H_2O	$C_{14}H_{19}NO$
9	1-Methyl-1-cyclopentyl	31–32.5	47	PE[b]	$C_{12}H_{17}NO$
10	1-Ethyl-1-cyclopentyl	Liquid[d]	58		$C_{13}H_{19}NO$
11	1-Methyl-1-cyclohexyl	53–55	53	PE	$C_{13}H_{19}NO$
12	1-Ethyl-1-cyclohexyl	Liquid[e]	57		$C_{14}H_{21}NO$
13	*tert*-Butyl	73–75[c]	95		$C_{10}H_{15}NO$

[a]Reduction step only. [b]Petroleum ether. [c]Lit.[8] mp 73-74°. [d]Distilled at 0.8 mm in a short-path oil-jacketed still; bath temperature 137°. [e]Distilled at 0.15 mm in a short-path oil-jacketed still; bath temperature 145-150°.

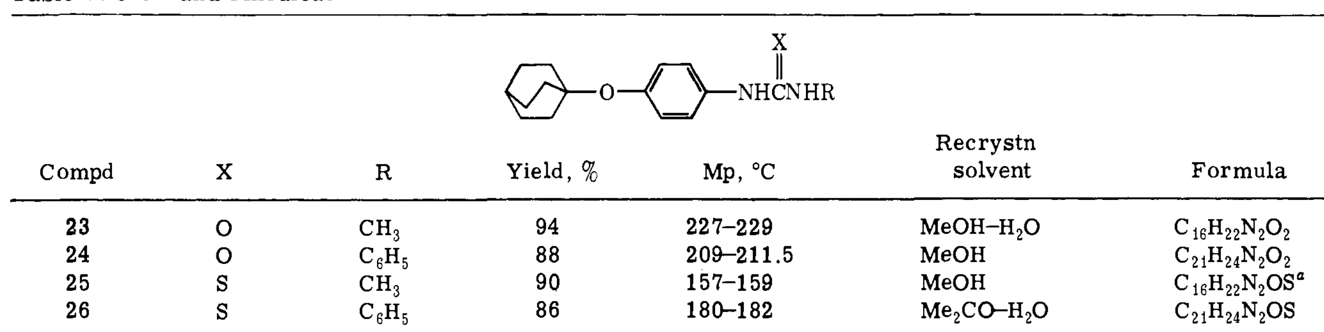
Table IV. *N*-Alkyl-*p*-(bicyclo[2.2.2]oct-1-yloxy)anilines



Compd	R	R′	Salt	Yield, %	Mp, °C	Recrystn solvent	Formula
17	H	CH_3	HCl	35	210–215	CH_2Cl_2–EtOAc	$C_{15}H_{22}ClNO$
19	H	C_2H_5	HCl	74	167–169	CH_2Cl_2–EtOAc	$C_{16}H_{24}ClNO$
20	H	$CH_2C_6H_5$		90	155–157	CH_2Cl_2–MeOH	$C_{21}H_{25}NO$
22	C_2H_5	C_2H_5	HCl	19[a]	234–237	CH_3CN	$C_{18}H_{28}ClNO$

[a]There was recovered 17% of deacylated product 19.

Table V. Ureas and Thioureas



Compd	X	R	Yield, %	Mp, °C	Recrystn solvent	Formula
23	O	CH_3	94	227–229	MeOH–H_2O	$C_{16}H_{22}N_2O_2$
24	O	C_6H_5	88	209–211.5	MeOH	$C_{21}H_{24}N_2O_2$
25	S	CH_3	90	157–159	MeOH	$C_{16}H_{22}N_2OS$[a]
26	S	C_6H_5	86	180–182	Me_2CO–H_2O	$C_{21}H_{24}N_2OS$

[a]Anal. C: calcd, 66.17; found, 67.59.

sulfamide (28). *N,N*-Dimethylsulfamyl chloride (1.10 g) was added cautiously to a solution of 1.50 g (6.9 mmol) of the aniline in 15 ml of pyridine. Following 15 hr of standing at room temperature, the bulk of the solvent was removed at 5 mm. The residue was treated with water and the solid dissolved in methylene chloride–chloroform. The solution was washed in turn with 2.5 *N* HCl, water, and brine and taken to dryness. The residual dark red solid was chromatographed on 200 ml of Florisil (elution with 20% acetone in Skellysolve B). The crystalline fractions were combined and recrystallized twice from aqueous methanol to give 1.52 g (72%) of product, mp 182–183°. Anal. ($C_{16}H_{24}N_2O_2S$) C, H, N.

Diazotization of *p*-(Bicyclo[2.2.2]oct-1-yloxy)aniline. A mixture of 2.0 g of the amine in 10 ml of sulfuric acid and 20 ml of water was warmed on the steam bath for 40 min. The slurry was cooled to 0° and a solution of 0.9 g of sodium nitrite in 8 ml of water added in portions over 20 min. The solution was then quickly filtered and treated in the cold with 2 ml of fluoroboric acid. The precipitate was collected on a filter, pressed dry, and suspended in 20 ml of acetic acid. The mixture was then heated for 30 min on the steam bath and 30 min at reflux. The solvent was removed in vacuo and the residue dissolved in ether. The solution was washed in turn with aqueous $NaHCO_3$, water, and brine. The residue which remained when the solvent was removed was chromatographed on 150 ml of Florisil. Elution with 10% acetone in Skellysolve B gave 0.70 g of hydroquinone, mp 168–170° (mmp with authentic material 168–170°) followed by 0.65 g of volatile solid whose NMR is identical with that of bicyclo[2.2.2]octan-1-ol.

Bicyclo[2.2.2]oct-1-yl *p*-Iodophenyl Ether (29). A solution of 11.1 g (0.051 mol) of the amine in 250 ml of THF was added to 7.15 g of amyl nitrite and 7.0 g of iodine in 150 ml of benzene over 20 min. The dark mixture was stirred at room temperature for 1 hr and at reflux for 2 hr. The mixture was taken to dryness and the residue chromatographed on 1 l. of silica gel (elution with 20% CH_2Cl_2 in Skellysolve B). There was obtained first 1.35 g of solid which consists largely of bicyclo[2.2.2]oct-1-yl phenyl ether, followed by the crude crystalline iodo compound. This was recrystallized from methanol to give 7.45 g (46%) of product, mp 71–73°. Anal. ($C_{14}H_{17}IO$) C, H.

***p*-(Bicyclo[2.2.2]oct-1-yloxy)benzoic Acid (30).** A solution of 3.0 g (9.3 mmol) of the iodide in 50 ml of THF in Dry Ice–acetone bath was treated with 6.4 ml of 1.6 *N* butyllithium. Following 1 hr of stirring in the cold, the mixture was transferred under N_2 onto finely crushed Dry Ice. The solid was then allowed to evaporate and the mixture taken to dryness. The residue was suspended in water and extracted with ether. The organic layer was washed twice with 1 *N* NaOH. Acidification of the combined aqueous layers afforded 1.21 g of acid, mp 182–193°. This was recrystallized twice from aqueous methanol to give 0.93 g (41%) of product, mp 199.5–201°. Anal. ($C_{15}H_{18}O_3$) C, H.

***p*-(Bicyclo[2.2.2]oct-1-yloxy)benzylamine Hydrochloride**

(32). A mixture of 2.88 g (12 mmol) of the acid, 10 ml of $SOCl_2$, and 40 ml of benzene was heated at reflux for 4 hr. The solvent was then removed in vacuo. The oily residue was dissolved in 25 ml of THF and added to 50 ml of THF saturated with NH_3; NH_3 was bubbled through for an additional hour and the mixture taken to dryness. The residue was suspended in water, collected on a filter, and then recrystallized from aqueous methanol. There was obtained 2.30 g of the amide 31, mp 173–177°.

A solution of the amide obtained above in 75 ml of THF was added to 1.0 g of $LiAlH_4$ in 10 ml of THF. The mixture was heated at reflux for 3 hr, cooled in ice, and treated in turn with 1 ml of H_2O, 1 ml of 15% NaOH, and 3 ml of H_2O. The gel was removed by filtration and the filtrate taken to dryness. The residue was dissolved in ether (30 ml) and treated with 10 ml of 3.7 *N* ethereal HCl. The precipitated solid was recrystallized from methanol to afford 1.82 g (57%) of product, mp 290–292°. Anal. ($C_{15}H_{22}ClNO$) C, H.

1-Methyl-4-[*p*-(bicyclo[2.2.2]oct-1-yloxy)phenyl]-4-hydroxypiperidine (33). Butyllithium (6.5 ml of 1.55 *N*) was added to a solution of 3.28 g (1.0 mmol) of the iodo compound in a Dry Ice–acetone bath. Following 2 hr of stirring in the cold there was added 1.2 g of 1-methyl-4-piperidone in 20 ml of THF. The mixture was allowed to stand at room temperature overnight. The solvent was removed in vacuo and the residue dissolved in ether and water. The organic layer was then extracted with four 40-ml portions of 2.5 *N* HCl. The last extracts were made strongly basic and extracted with ether. The ethereal extract was worked up in the usual way. The residue was recrystallized from ether-Skellysolve B to afford 0.20 g (6.3%) of product, mp 137–139°. Anal. ($C_{20}H_{29}NO_2$) C, H.

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Nucleic Acid Related Compounds. 17. 3-Deazauridine. Stannous Chloride Catalysis of *cis*-Diol vs. Phenolic Base Methylation with Diazomethane[1]

Morris J. Robins* and Alan S. K. Lee

Department of Chemistry, The University of Alberta, Edmonton, Alberta, Canada T6G 2G2.

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Treatment of a methanolic solution of 4-hydroxy-1-β-D-ribofuranosyl-2-pyridinone (3-deazauridine, 1) with diazomethane gave 2-methoxy-1-β-D-ribofuranosyl-4-pyridinone (2) and 4-methoxy-1-β-D-ribofuranosyl-2-pyridinone (3a) in an approximate ratio of 1:2. Analogous treatment of 1 with diazomethane in the presence of stannous chloride dihydrate gave eight detected products including **2**, 2-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-4-pyridinone (4), 2-methoxy-1-(3-*O*-methyl-β-D-ribofuranosyl)-4-pyridinone (**5**), **3a**, 4-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyridinone (**6a**), 4-methoxy-1-(3-*O*-methyl-β-D-ribofuranosyl)-2-pyridinone (**7a**), 2'-*O*-methyl-3-deazauridine (**6b**), and 3'-*O*-methyl-3-deazauridine (**7b**). For comparison, the 2'-*O*- and 3'-*O*-methyl derivatives of 2 (4 and 5) and of 3a (6a and 7a), respectively, were prepared in good overall yields by stannous chloride catalyzed methylation of 2 and 3a. Treatment of 1 with benzyl bromide gave 4-benzyloxy-1-β-D-ribofuranosyl-2-pyridinone (**3b**). Stannous chloride catalyzed methylation of 4-pivaloxy-1-β-D-ribofuranosyl-2-pyridinone (**3c**) gave the corresponding 2'-*O*-methyl derivative **6c**. These compounds were tested in leukemia L1210 culture and against three bacterial strains and were found to be uniformly inactive. This provides a striking example of nucleoside structure specificity and also adds support to the depot storage–enzymic cleavage mode of antileukemic activity of 4-(adamantane-1-carbonyloxy)-1-β-D-ribofuranosyl-2-pyridinone (**3d**).

The pyridine nucleus analog of uridine, 4-hydroxy-1-β-D-ribofuranosyl-2-pyridinone (3-deazauridine, 1), was synthesized[2a] in order to examine the biological effects of such a formal replacement of -NHCO- by -CH=COH- at positions 3 and 4 in the pyrimidine system. Both 1 and the corresponding 3-deazacytidine[2b] (4-amino-1-β-D-ribofuranosyl-2-pyridinone) were found to exert marked inhibitory effects on the growth of neoplastic and bacterial cultures.[3] A number of other 3-deaza analogs of the pyrimidine 2'-deoxy nucleosides, arabinosides, orotic acid, etc., were subsequently prepared.[4] However, little activity was noted with these compounds.[5] Heidelberger and coworkers and Shone have recently extended this concept to the preparation of pyridine analogs of 5-fluorouracil and thymine bases, nucleosides, and 2'-deoxy nucleosides.[6] Again, unfortunately, biological activity was found to be lacking.[6a] The lone example of retained (and, indeed, enhanced) activity in a modified 3-deazauracil nucleoside involved esterification of the phenolic 4-oxygen of 3-deazauridine with adamantane-1-carboxylic acid.[5] However, evidence was presented which was compatible with enzymatic hydrolysis of the ester function, making this derivative (**3d**) a depot-storage form of 3-deazauridine (1) per se.

It has been found very recently[7] that 3-deazauridine has markedly enhanced activity in leukemia strains which have become resistant to 1-β-D-arabinofuranosylcytosine (ara-C). Toxicity levels appear manageable (Dr. A. Bloch, private communication) and clinical trials appear to be warranted. Bloch and coworkers[8] have now reported that 3-deazauridine 5'-triphosphate inhibits the mammalian enzyme cytidine triphosphate synthetase, and this may be the major site of action. This, coupled with the observed enzymatic phosphorylation of 1 to the triphosphate level but lack of incorporation into nucleic acids,[9] might rationalize