Typical experiments are summarized in Tables I and II. The results of a series of experiments are listed in Table III.

Registry No.—(±)-**3**, 30256-03-2; (−)-**3**, 30318-67-3; (±)-**4**, 30256-04-3; (±)-**5**, 30247-99-5; (+)-**5**, 30248-00-1; (±)-**6**, 30275-69-5; (+)-**6**, 30248-01-2; (±) inactive polymer, 30228-77-4; (+) inactive polymer, 30228-78-5.

Synthesis of 2- and 3-Keto-5-*endo*-(2-imidazolyl)bicyclo[2.2.2]octane

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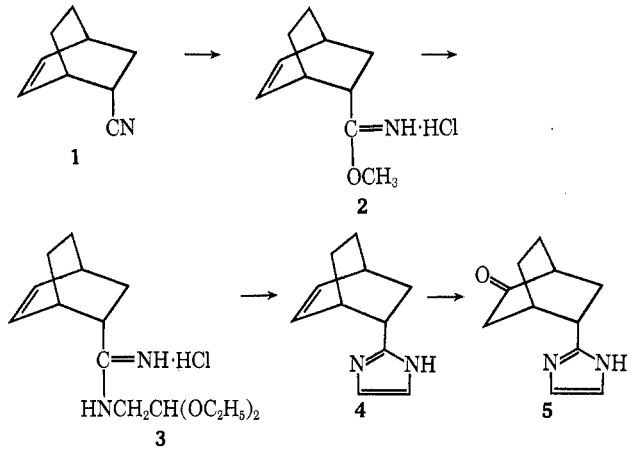
On the basis of a concept of attributes that make compounds attractive candidates for biological screening, 2-keto- and 3-keto-5-*endo*-(2-imidazolyl)bicyclo[2.2.2]octane were synthesized on a relatively large scale for conversion to a series of corresponding 2- or 3-substituted analogs.

In order to raise the probability of finding biological activity among candidates for pharmacometric screening, a family of compounds with structural features that should *a priori* enhance their effectiveness was synthesized. Three features included in this particular series were (1) a rigid bicyclic framework for favorable entropy of binding to a receptor, (2) an imidazole nucleus, attractive for its bifunctional nature and participation at active sites of enzymes, and (3) a second functional group chosen from those found among naturally occurring compounds. Although the biological activity of the group was disappointing, certain aspects of this synthetic organic chemistry are worthy of note.

On the basis of the criteria outlined above, 5-*endo*-(2-imidazolyl)bicyclo[2.2.2]oct-2-ene (**4**) was selected as an appropriate starting compound. The most logical way to synthesize **4** is by a Diels–Alder condensation between cyclohexadiene-1,3 and a 2-vinylimidazole,[1] but this method was soon abandoned in favor of a stepwise synthesis starting from 5-*endo*-cyanobicyclo[2.2.2]-oct-2-ene.

The target compound **4** was synthesized starting with the conversion of 5-*endo*-cyanobicyclo[2.2.2]oct-2-ene[2] (**1**) to 5-*endo*-carbiminomethoxybicyclo[2.2.2]-oct-2-ene hydrochloride (**2**) on treatment with methanol and hydrogen chloride in ether solution. Reaction of the imino ether **2** with β-aminoacetaldehyde diethyl acetal yielded *N*-(β,β-diethoxyethyl)-5-*endo*-carbamidinobicyclo[2.2.2]oct-2-ene hydrochloride (**3**), which at 120° in glacial acetic acid–acetic anhydride gave 5-*endo*-(2-imidazolyl)bicyclo[2.2.2]oct-2-ene (**4**).

A critical step in the scheme required the introduction at an appropriate position on the bicyclic framework of a carbonyl group which could then be converted into a variety of functional groups. The unsaturation at the 2,3 position of the bicyclic skeleton was a logical point to attack, but more difficulty was encountered than anticipated in applying typical reactions for this transformation. The imidazole moiety appeared to be largely responsible for the difficulty by either preventing reaction at the 2 or 3 position or by being susceptible to attack by the reagent. For example, in a variety of hydroboration studies, stable aminoborane derivatives were isolated and the unsaturated moiety was intact. In other instances involving mild oxidizing agents, the imidazole ring was attacked. Facile conversion of the unsaturated compound **4** to 2-keto-5-*endo*-(2-imidazolyl)bicyclo[2.2.2]octane (**5**) was achieved by palladium chloride oxidation in aqueous medium.[3] The reaction is usually accomplished by using catalytic quantities of palladium chloride in the presence of large amounts of cupric chloride and an air stream. The propensity of the keto imidazole derivative **5** to chelate with copper, however, made it prudent to use palladium chloride in at least stoichiometric amount and eliminate the cupric salt.



The location of the carbonyl group in **5** could not be established on theoretical grounds and was not immediately obvious from nmr spectra data in $CDCl_3$. In the absence of model compounds, chemical shifts could not be exploited with assurance, since the positions of protons adjacent to the ketone would be similar in both the 2 and 3 isomers. Furthermore, any difference in multiplicity between the bridgehead protons could not be used as an approach to structure assignment because of the coincidence of the C_1 and C_4 proton peaks in several solvents. These difficulties were overcome by taking advantage of the long-range coupling

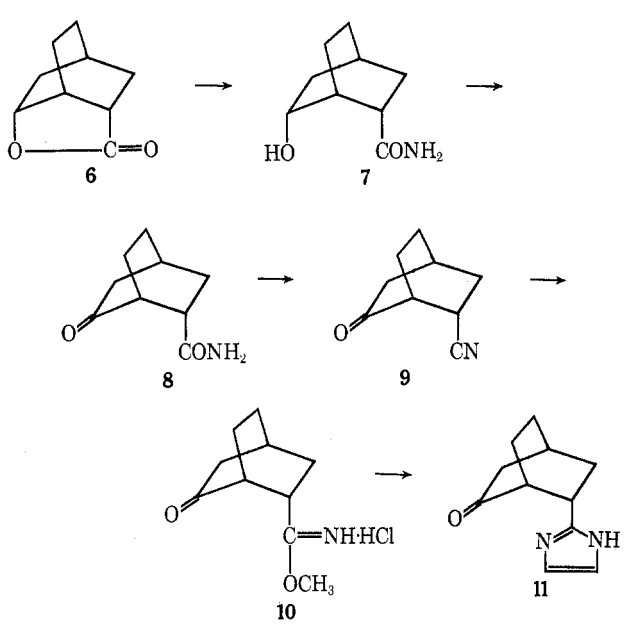
(1) The relatively poor dienophilic nature of vinylimidazoles and their propensity to polymerize at relatively low temperature did not augur well for a successful Diels–Alder condensation. In addition, considerable difficulty was justifiably anticipated in the prepartion of certain 2-vinylimidazoles on a reasonably large scale. Nevertheless, 1-benzyl-2-vinylimidazole was synthesized from 1-benzyl-2-lithioimidazole by reaction with acetaldehyde and dehydration over fused $KHSO_3$ at 251–230° (25–45 mm). The distilled product (*ca.* 60% pure) did not undergo Diels–Alder condensation with cyclohexadiene-1,3 at temperatures up to 170° or at pressures up to 138,000 psi. In a parallel attempt, methyl β-(4-imidazolyl)acrylate was prepared from L-histidine and heated with cyclohexadiene-1,3 at temperatures up to 210° without success.

(2) K. Alder, H. Heimbach, and R. Reubke, *Chem. Ber.*, **91**, 1516 (1958).

(3) For a review see J. Smidt, W. Hafner, R. Jira, R. Sieber, J. Sedlmeier, and A. Sabel, *Angew. Chem., Int. Ed. Engl.*, **1**, 80 (1962).

often observed between exo protons separated by four single bonds.[4] In the bicyclo[2.2.2]octane system, this would be reflected by coupling between the C_5 and C_3 exo protons. The detection of such interaction, most readily determined from a critical examination of the C_5 resonance, would therefore exclude the C_3 site for the ketone. In pyridine, the C_5 proton resonance is composed of 11 peaks. This is consistent with an overall multiplicity of 16 lines arising from coupling constants of 10.7, 6.0, 2.0, and 2.0 Hz. The observed pattern results from the coincidence of lines 2–3, 6–7, 8–9, 10–11, and 14–15. Since the three vicinal protons can account for a maximum of eight lines, it follows that the C_5 proton is involved in long-range coupling with a fourth proton, presumably the exo proton at C_3. Compound **5** was therefore assigned the 2-keto structure. This line of reasoning was later confirmed when it was shown that the C_5 proton resonance in the 3-keto isomer, prepared by an unambiguous synthesis, was composed of eight lines.

For the synthesis of the 3-keto analog **11**, bicyclo[2.2.2]oct-2-ene-5-carboxylic acid[5] served as the starting material. Treatment of this acid with 30% sulfuric acid at 110° for 1 hr[6] gave excellent yields of 3-*endo*-hydroxy-5-*endo*-carboxybicyclo[2.2.2]octane lactone (**6**), which on ammonolysis yielded 3-*endo*-hydroxy-5-*endo*-carbamylbicyclo[2.2.2]octane (**7**). Treatment of the hydroxy analog **7** with chromic acid in acetic acid gave the corresponding keto derivative **8**, which on dehydration with *p*-toluenesulfonyl chloride in pyridine



yielded 3-keto-5-*endo*-cyanobicyclo[2.2.2]octane (**9**). The cyano derivative **9** was converted to 3-keto-5-*endo*-(2-imidazolyl)bicyclo[2.2.2]octane (**11**) by way of the imino ether **10**.

(4) F. A. L. Anet, *Can. J. Chem.*, **39**, 789 (1961); T. F. Flautt and W. F. Erman, *J. Amer. Chem. Soc.*, **85**, 3212 (1963); J. Meinwald and Y. Meinwald, *ibid.*, **85**, 2514 (1963); K. Tori, Y. Takano, and K. Kitahonoki, *Chem. Ber.*, **97**, 2798 (1964).

(5) R. Seka and O. Tramposh, *ibid.*, **75**, 1379 (1942).

(6) These conditions are superior to those reported by K. Alder and G. Stein, *Justus Liebigs Ann. Chem.*, **514**, 197 (1934), or W. R. Boehme, E. Schipper, W. G. Scharf, and J. Nichols, *J. Amer. Chem. Soc.*, **80**, 5488 (1958).

Experimental Section[7]

5-*endo*-Carbiminomethoxybicyclo[2.2.2]oct-2-ene Hydrochloride (2).—A solution of 2 g (62 mmol) of MeOH and 8.3 g (62 mmol) of 5-*endo*-cyanobicyclo[2.2.2]oct-2-ene[3] in 18 ml of anhydrous Et_2O was cooled, and 2.3 g of anhydrous HCl in Et_2O was added. After being allowed to stand overnight at 5°, the mixture was filtered, yielding 6.6 g of **2**, mp 165–167°. *Anal.* Calcd for $C_{10}H_{16}ClNO$: C, 59.55; H, 8.00; Cl, 17.58; N, 6.95. Found: C, 59.86; H, 8.16; Cl, 17.46; N, 7.17.

***N*-(β,β-Diethoxyethyl)-5-*endo*-carbamidinobicyclo[2.2.2]oct-2-ene Hydrochloride (3).**—A suspension of 40 g (0.2 mol) of **2** in 87 ml of MeOH was warmed to 30° and 27.4 g (0.21 mol) of β-aminoacetaldehyde diethyl acetal was added rapidly. The temperature of the suspension rose to 51°, and the mixture became clear. After 1 hr, the solution was concentrated under reduced pressure, yielding 62.3 g of **3**.

5-*endo*-(2-Imidazolyl)bicyclo[2.2.2]oct-2-ene (4).—A solution of 62.3 g (0.2 mol) of **3** in 260 ml of AcOH and 111 ml of Ac_2O was heated at 120° for 2 hr. The reaction mixture was concentrated under reduced pressure, and the product was dissolved in 270 ml of 2.5 *N* HCl. The acidic solution was washed with Et_2O, made alkaline with 50% KOH, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was taken up in a small volume of $CHCl_3$ and adsorbed on 500 g of silica gel packed in CH_2Cl_2. The product was eluted with 0.5% MeOH in CH_2Cl_2 and recrystallized from 100 ml of C_6H_6–petroleum ether (bp 30–60°) yielding 20.95 g of **4**, mp 165–167°. *Anal.* Calcd for $C_{11}H_{14}N_2$: C, 75.82; H, 8.10; N, 16.08. Found: C, 76.32; H, 7.83; N, 15.83. Repeated crystallization of the product failed to improve the elemental analysis. Accordingly, 13.5 g of **4** was treated with 6 ml of methyl chloroformate and 32 g of K_2CO_3 in 400 ml of Me_2CO. The mixture was filtered and concentrated, and an Et_2O-soluble fraction was isolated by trituration of the residue. Concentration of the Et_2O solution yielded 11.8 g of the 1-carbomethoxy analog of **4**, which on treatment with 250 ml of 1 *N* HCl yielded 7.8 g of **4**. Passage of this material through 10 g of silica gel as above yielded 6.2 g of **4**, mp 163–164°. *Anal.* Calcd for $C_{11}H_{14}N_2$: C, 75.82; H, 8.10; N, 16.08. Found: C, 75.66; H, 7.81; N, 15.95.

2-Keto-5-*endo*-(2-imidazolyl)bicyclo[2.2.2]octane (5).—A mixture of 14.5 g (83 mmol) of **4**, 10 g of $PdCl_2$, and 14.5 g of $CuCl_2 \cdot 2H_2O$ in 170 ml of 1 *N* HCl and 900 ml of H_2O was stirred and heated at 70–80° for 1 hr while air was bubbled through the solution continuously.[8] The hot solution was filtered and cooled, and 140 g of Na_4EDTA was added. The solution was extracted five times with $CHCl_3$, and the combined extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure, yielding an 8.2-g residue. The product was adsorbed on silica gel packed in CH_2Cl_2, and the column was developed slowly with 0–1% MeOH in CH_2Cl_2. The product was eluted with 1–2% MeOH in CH_2Cl_2 and recrystallized from $CHCl_3$–Et_2O, yielding 7.9 g of **5**, mp 194–196°. *Anal.* Calcd for $C_{11}H_{14}N_2O$: C, 69.44; H, 7.42; N, 14.73. Found: C, 69.73; H, 7.71; N, 14.71

For a more readily purified product the following alternative procedure may be used. A mixture of 49 g of $PdCl_2$ in 250 ml of 1 *N* HCl was warmed to 180°, diluted with 2250 ml of hot H_2O, and heated under reflux. Next, 44 g of **4** was dissolved in 250 ml of warm 1 *N* HCl, and the solution was diluted with 800 ml of warm H_2O. The solution of **4** was added to the refluxing $PdCl_2$ mixture in the course of 0.5 hr, and the mixture was stirred under reflux for 8 hr. The mixture was filtered and the filtrate was concentrated to a 700-ml volume. The pH of the concentrate was adjusted to 8 with 30% NH_4OH and the product was extracted continuously with $CHCl_3$. Concentration of the $CHCl_3$ extract yielded 39.3 g (82%) of product, which is readily purified by chromatography on silica gel (above) without prior slow development, yielding 8.8 g of starting material and 29.0 g of **5**, mp 199–200°. *Anal.* Calcd for $C_{11}H_{14}N_2O$: C, 69.44; H, 7.42; N, 14.73. Found: C, 69.53; H, 7.49; N, 14.65.

(7) Melting points were determined on a Koefler hot stage and are uncorrected. The ir spectra of all the compounds were consistent with the assigned structures. The nmr spectra were obtained on Varian A-60 and HA-100 spectrometers; concentrations were generally 5–10% (*w*/*v*), although a few samples were examined in the 10–20% range on the lower frequency instrument.

(8) The $CuCl_2 \cdot 2H_2O$ and air are used to regenerate $PdCl_2$ but complicate purification of the product. It is more convenient to use an excess of $PdCl_2$ and omit the $CuCl_2 \cdot H_2O$ and air flow.

3-*endo*-Hydroxy-5-*endo*-carboxybicyclo[2.2.2]octane Lactone (6).—The published methods[6] were modified as follows. Bicyclo-[2.2.2]oct-2-ene-5-carboxylic acid[5,6] (100 g) in 720 ml of 30% (v/v) H_2SO_4 was stirred and heated at 110° for 1 hr. The mixture was cooled, poured onto ice, and extracted with $CHCl_3$. The extract was washed with 10% $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure, yielding 74.3 g of **6**, mp 207–208°.

3-*endo*-Hydroxy-5-*endo*-carbamylbicyclo[2.2.2]octane (7).—A solution of 500 mg of **6** in 10 ml of MeOH and 10 ml of liquid NH_3 was heated at 110° for 12 hr. The reaction mixture was concentrated under reduced pressure, and the residue was recrystallized from hot $CHCl_3$–petroleum ether, yielding 500 mg of **7**, mp 188.5–189°. *Anal.* Calcd for $C_9H_{15}NO_2$: C, 63.88; H, 8.94; N, 8.28. Found: C, 64.09; H, 9.24; N, 8.50.

3-Keto-5-*endo*-carbamylbicyclo[2.2.2]octane (8).—A solution of 28.6 g of CrO_3 in 358 ml of 90% AcOH was added dropwise in the course of 2 hr to a stirred solution of 40 g of **7** in 385 ml of AcOH. After being stirred overnight, the mixture was concentrated under reduced pressure, diluted with 300 ml of H_2O, and extracted continuously overnight with $CHCl_3$. The extract was dried over $MgSO_4$, filtered, and concentrated under reduced pressure, yielding 26.3 g of crystallized product that was used in the next step. Recrystallization of a small sample of the product from MeOH–Et_2O gave **8**, mp 183–184°. *Anal.* Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.63; H, 7.93; N, 8.57.

3-Keto-5-*endo*-cyanobicyclo[2.2.2]octane (9).—A mixture of 1.15 g (7 mmol) of **8** and 1.45 g (7.7 mmol) of TsCl in 5 ml of pyridine was heated at 80° for 2 hr. The mixture was diluted with 5 ml of H_2O and concentrated; the residue as taken up in $CHCl_3$, and the solution was concentrated. After the residue was taken up in $CHCl_3$ and the solution was concentrated a second time, the same procedure was repeated with C_6H_6. The residue was then triturated with C_6H_6, and the mixture was filtered. The C_6H_6 extract was concentrated and the residue was recrystallized from $CHCl_3$–petroleum ether, yielding 0.55 g of **9**, mp 145–152° (subl 90–130°). *Anal.* Calcd for $C_9H_{11}NO$: C, 72.45; H, 7.43; N, 9.39. Found: C, 72.52; H, 7.36; N, 9.44.

3-Keto-5-*endo*-carbiminomethoxybicyclo[2.2.2]octane Hydrochloride (10).—A solution of 250 mg of **9** and 100 mg of MeOH in 5 ml of Et_2O was cooled and saturated with anhydrous HCl. The next day, the mixture was poured into 100 ml of Et_2O and filtered, yielding 220 mg of **10**, mp 170–175°.

3-Keto-5-*endo*-(2-imidazolyl)bicyclo[2.2.2]octane (11).—A solution of 107 g (0.49 mol) of **10** in 240 ml of MeOH was added dropwise in the course of 45 min to 144 g (1.08 mol) of β-aminoacetaldehye diethyl acetal at 55–60°. The mixture was stirred at room temperature for 1 hr and concentrated under reduced pressure, yielding a 239-g residue. The product was dissolved in 1 l. of 6 *N* HCl and heated under reflux for 1 hr. The solution was cooled, extracted with $CHCl_3$, made alkaline with concentrated NH_4OH, and extracted with $CHCl_3$. Concentration of the latter yielded a 60-g residue that was purified by adsorption on 550 g of silica gel packed in CH_2Cl_2, elution with 4% MeOH–CH_2Cl_2, and recrystallization from CH_2Cl_2–Et_2O, yielding 37.1 g of **11**, mp 144–147°. *Anal.* Calcd for $C_{11}H_{14}N_2O$: C, 69.44; H, 7.42; N, 14.73. Found: C, 69.63; H, 7.49; N, 14.70.

Registry No.—**2**, 30338-51-3; **4**, 30338-52-4; **5**, 30338-53-5; **6**, 20507-79-3; **7**, 30338-55-7; **8**, 30338-56-8; **9**, 30338-57-9; **10**, 30338-58-0; **11**, 30338-59-1.

Acknowledgment.—We are indebted to Mr. A. P. Sullivan, Jr., for developing the $PdCl_2$ oxidation of **4** to **5** in the absence of copper salts and to Mr. R. N. Boos and associates for elemental analyses.

Tumor Inhibitors. LXV.[1] Bersenogenin, Berscillogenin, and 3-Epiberscillogenin, Three New Cytotoxic Bufadienolides from *Bersama abyssinica*[2]

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An ethanol extract of the fruits of *Bersama abyssinica* was found to show significant inhibitory activity against cells derived from human carcinoma of the nasopharynx carried in cell culture (KB). The isolation and structural elucidation of three new cytotoxic bufadienolides, berscillogenin (**1**), 3-epiberscillogenin (**2**), and bersenogenin (**3**), are reported. Mass spectrometry and elemental analysis indicated that all three compounds had a $C_{24}H_{30}O_6$ molecular formula. Chemical and spectral evidence support assignment of structure **1** (16β-hydroxyscilloglaucosidin) for berscillogenin. The same enone (**7**) was obtained from manganese dioxide oxidation of **1** and **2**, indicative that the compounds are C-3 epimers. Treatment of **3** with 80% acetic acid afforded both **1** and **2**. This reaction and the nmr spectrum of **3** indicated that it is a Δ^3-5β-hydroxy isomer of berscillogenin (**1**). The isolation and identification of hellebrigenin 3-acetate (**4**) and scilliglaucosidin (**5**) are also discussed.

In the course of a continuing search for tumor inhibitors of plant origin,[4] we found that extracts of the fruits of *Bersama abyssinica* Fresen. (*Melianthaceae*)[5] showed significant inhibitory activity against cells derived from human carcinoma of the nasopharynx carried in cell culture (KB).[6] Previously, we have reported systematic studies of the KB-inhibitory principles of the stem bark of *B. abyssinica* which led to the isolation and structural elucidation of the cytotoxic principles, hellebrigenin 3-acetate and hellebrigenin 3,5-diacetate,[7] as well as four novel naturally occurring bufadienolide orthoacetates and two related acetate esters.[1] The observation that hellebrigenin 3-acetate showed significant activity *in vivo* against the Walker intramuscular carcinosarcoma 256 in rats stimulated further

(1) Part LXIV: S. M. Kupchan, I. Ognyanov, and J. L. Moniot, *Bioorg. Chem.*, in press.

(2) This investigation was supported by grants from the National Institutes of Health (HE-12957 and CA-11718) and the American Cancer Society (T-275) and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH-43-64-551). C. W. S. was a NIH Postdoctoral Fellow, 1967–1969.

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(4) S. M. Kupchan, *Trans. N. Y. Acad. Sci.*, **32**, 85 (1970).

(5) Fruits of *B. abyssinica* were collected in Ethiopia, Jan 1968. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with USDA by the Cancer Chemotherapy National Service Center (CCNSC).

(6) Cytotoxicity was assayed under the auspices of the CCNSC and the procedures were those described in *Cancer Chemother. Rep.*, **25**, 1 (1962). Cytotoxicity was also assayed by differential agar diffusion by Professor D. Perlman, University of Wisconsin; *cf.* D. Perlman and J. L. Schwartz, *J. Pharm. Sci.*, **58**, 633 (1969).

(7) S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, *J. Org. Chem.*, **34**, 3894 (1969).