Cyclopropylogs of Thyronine Derivatives[1a]

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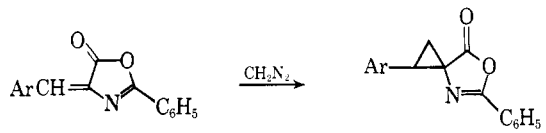
The synthesis of 1-amino-*trans*-2-[4-(4-hydroxyphenoxy)-3,5-diiodophenyl]cyclopropanecarboxylic acid has been performed, the key step being the addition of CH_2 (from diazomethane) to the exocyclic double bond of the azlactone, 4-[4-(4-acetoxyphenoxy)-3,5-diiodobenzylidene]-2-phenyl-2-oxazolin-5-one. The stereochemistry of the amino acid has been established. The 3′,5′-dibromo derivative exhibited weak thyromimetic activities in basal metabolism and antigoiter tests and did not inhibit increased metabolism developed by triiodothyronine.

As part of our program to study biologically interesting amines and amino acids with sterically hindering groups adjacent to the amino and/or carboxyl functions,[2,3] we are paying particular attention to analogs in which the α-carbon atom is part of a small ring.[4-7] Such compounds have been shown to inhibit the enzymatic degradation of some of their natural metabolite prototypes; for the activity of small α-alkyl-substituted analogs of further amino acids, see ref 8–10. We now describe the synthesis, stereochemistry, and biological properties of cyclopropylogs of thyronines related to the natural thyroid hormones.

After exploring several approaches to the synthesis of 1-amino-2-arylcyclopropanecarboxylic acids,[11,12] we found what appeared to be a very promising pathway in the reaction of azlactones[13] with diazomethane; the resulting 1-aryl-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-ones (*e.g.*, XII)[14] appeared to be likely intermediates for the preparation of the corresponding cyclopropaneamino acids. Consequently, 4-[4-(4-acetoxyphenoxy)-3,5-diiodobenzylidene]-2-phenyl-2-oxazolin-5-one (V) was treated with diazomethane and yielded 1-[4-(4-acetoxyphenoxy)-3,5-diiodophenyl]-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-one (VI), which on reflux with hydrochloric acid gave the 3,5-diiodothyronine analog VII.



XII, Ar = 4-$CH_3OC_6H_4$

The azlactone V was obtained from the 4′-methoxyphenoxy-3,5-diiodo azlactone IV which had been used by Harington and Barger in their classical synthesis of thyroxine.[15] We prepared IV by a more convenient route from *p*-hydroxybenzaldehyde in five steps in an over-all yield of 17.5% as shown in Chart I. At this point the 4′-methoxy group had to be removed because it was expected that ether cleavage with HBr subsequent to the construction of the cyclopropane ring might disrupt this strained portion of the molecule. This was achieved by treatment of IV with boron tribromide[16] followed by acetylation of the reaction mixture.[17]

Many attempts to iodinate VII with I_2–KI in ammonium hydroxide or aqueous methylamine, or with iodine monochloride in acetic acid–20% HCl were unsuccessful, a low degree of iodination being achieved at best; VII was recovered essentially unchanged from these experiments. Since 3′ and/or 5′ substitution is known to be essential for significant thyromimetic activity, VII was brominated in acetic acid; the corresponding 3′,5′-dibromo compound (VIII) was obtained without difficulty. As shown later in this paper, VIII was recognized as 1-amino-*trans*-2-[4-(3,5-dibromo-4-hydroxyphenoxy)-3,5-diiodophenyl]-cyclopropanecarboxylic acid.

In order to test VIII for biological activity,[18] the following experiments were carried out: tests for the effects of graded doses of VIII on oxygen consumption and heart rate in normal and thyroidectomized rats, antigoiter and antimetabolism tests in rats. The oxygen consumption and heart rates were followed in a series of rats at the dosage levels of 1.14, 0.69, and 0.23 mg of VIII/100-g rat injected daily intraperitoneally for a period of 11 days followed by an 8-day period of recovery. At the same time, a comparable study was made by injecting daily 8.25 μg of triiodo-L-thyronine (T-3)/100-g rat for a similar period of time. At each dosage level, three rats were used except at the 1.14-mg level when four rats were employed. There was a relationship between metabolic response and dosage, for after 7 days of injecting VIII, the metabolism on 0.23 mg of drug had increased 33%, on 0.69 mg, 81%, and on 1.14 mg, 100%. After 8 days on T-3, the metabolism rose 107%. Therefore, VIII increases the oxygen consumption of thyroidectomized

(1) (a) Supported in part by Grant GM-12781 from the Institute of General Medicine, National Institutes of Health, U. S. Public Health Service. (b) Du Pont Teaching Fellow, 1963–1964; NASA Trainee, 1965–1966.

(2) A. Burger, S. E. Zimmerman, and E. J. Ariens, *J. Med. Chem.*, **9**, 469 (1966).

(3) A. Parulkar, A. Burger, and D. Aures, *ibid.*, **9**, 738 (1966).

(4) R. A. Pages and A. Burger, *ibid.*, **9**, 766 (1966).

(5) (a) A. Burger and W. E. Coyne, *J. Org. Chem.*, **29**, 3079 (1964); (b) A. Burger and S. E. Zimmerman, *Arzneimittel-Forsch.*, **16**, 1571 (1966).

(6) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. E. Tedeschi, and A. Burger, *J. Med. Pharm. Chem.*, **5**, 1265 (1962), and references cited therein.

(7) C. Beard and A. Burger, *J. Org. Chem.*, **26**, 2335 (1961); **27**, 1647 (1962).

(8) A. R. Patel and A. Burger, *Progr. Drug Res.*, **9**, 229 (1966).

(9) G. Kahlson, E. Rosengren, and R. Thunberg, *J. Physiol.* (London), **169**, 467 (1963).

(10) B. Blank, E. G. Rice, F. R. Pfeiffer, and C. M. Greenberg, *J. Med. Chem.*, **9**, 10 (1966), and ref 1–4 therein.

(11) C. Kaiser and C. L. Zirkle, Belgian Patent 648,020 (Nov 16, 1964).

(12) A. Burger and A. R. Patel, unpublished results.

(13) H. E. Carter, *Org. Reactions*, **3**, 198 (1946); E. Baltazzi, *Quart. Rev.* (London), **9**, 150 (1955).

(14) W. I. Awad, A. K. Fateen, and M. Zayed, *Tetrahedron*, **20**, 891 (1964).

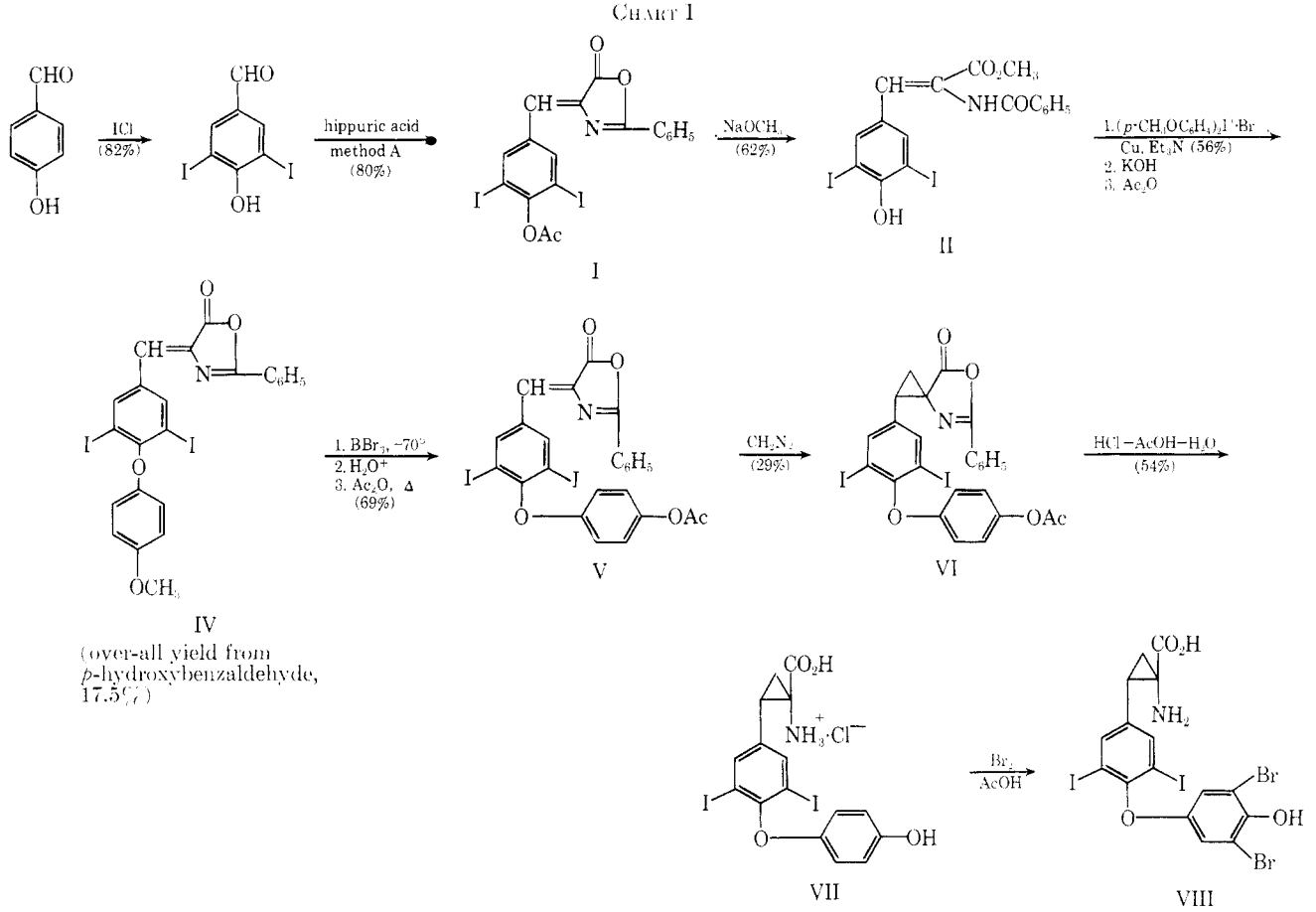
(15) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

(16) F. L. Benton and T. E. Dillon, *J. Am. Chem. Soc.*, **64**, 1128 (1942); S. Allen, T. G. Bonner, E. J. Bourne, and N. M. Saville, *Chem. Ind.* (London), 630 (1958); J. F. W. McOmie and M. L. Watts, *ibid.*, 1658 (1963).

(17) The infrared spectrum of this reaction mixture indicated that not only was the methoxy group cleaved but some of the azlactone ring had been opened with the formation of the α-benzamidocarboxylic acid.

(18) The biological tests were carried out in the Department of Pharmacology, University of Virginia School of Medicine, by Chalmers L. Gemmill and Katherine Mayo Browning.

CHART I



(over-all yield from *p*-hydroxybenzaldehyde, 17.5%)

rats but its activity is $^1/_{140}$ that of T-3. There was very little, if any, biological activity at the same dosage levels in normal rats. The heart rates showed changes comparable to those of the basal metabolism in the thyroidectomized rats.

In the antigoiter test, the rats were given 0.13 mg % of methimazole in their drinking water. In a group of 13 rats, 8.25 μg of T-3 was given intraperitoneally daily for 8 days. In 14 rats, 1.14 mg of VIII was given daily for 8 days. A group of six rats was only given methimazole. The thyroid weights of the controls averaged 4.5 mg/100-g rat, the T-3 group averaged 7.2 mg, the group receiving VIII averaged 9.6 mg, while the methimazole group averaged 13.9 mg. Again, it is clear that VIII does have weak biological activity.

In the antimetabolism test, the metabolism of eight rats was followed on 8.3 μg of T-3, and on 8.3 μg of T-3 plus 1.16 mg of VIII intraperitoneally for a period of 11 days. At this time the groups were changed, the T-3 group now receiving VIII, and the T-3 plus VIII group only receiving T-3. There was a slight lag in the increment of metabolism when the rats received both compounds but the metabolism of the T-3 group was not diminished on addition of VIII. Therefore, VIII does not have an inhibitory action on the increased metabolism developed by T-3.

By way of comparison, the 3′,5′-dibromo analog of (±)-thyroxine has about 1% the effect of thyroxine on the oxygen consumption of rats.[19]

Stereochemistry.—As a basis for the exploration of the possible relationship between the configuration of the cyclopropaneamino acid VIII to its biological activity, the stereochemistry of VI had to be elucidated. It should be remarked that the geometry of unsaturated azlactones such as V is not known with certainty. The condensation of an aldehyde with hippuric acid by the Erlenmeyer–Plöchl synthesis generally yields only one stereoisomer,[20] presumably the more stable one.[4,13] Depending on its mode of addition, diazomethane could add to this homogeneous stereoisomer to furnish spirooxazolones in which the carbonyl group of the oxazolone ring is *cis* and/or *trans* to the 1-aryl moiety. In all cases so far studied in this and previous papers[4,14,21] only one isomer has been isolated. A definite assignment of configuration of VI has now been made: the carbonyl group of its oxazolone ring is *trans* to the 4′-acetoxyphenoxy-3,5-diiodophenyl group based on the following considerations (see also Chart II).

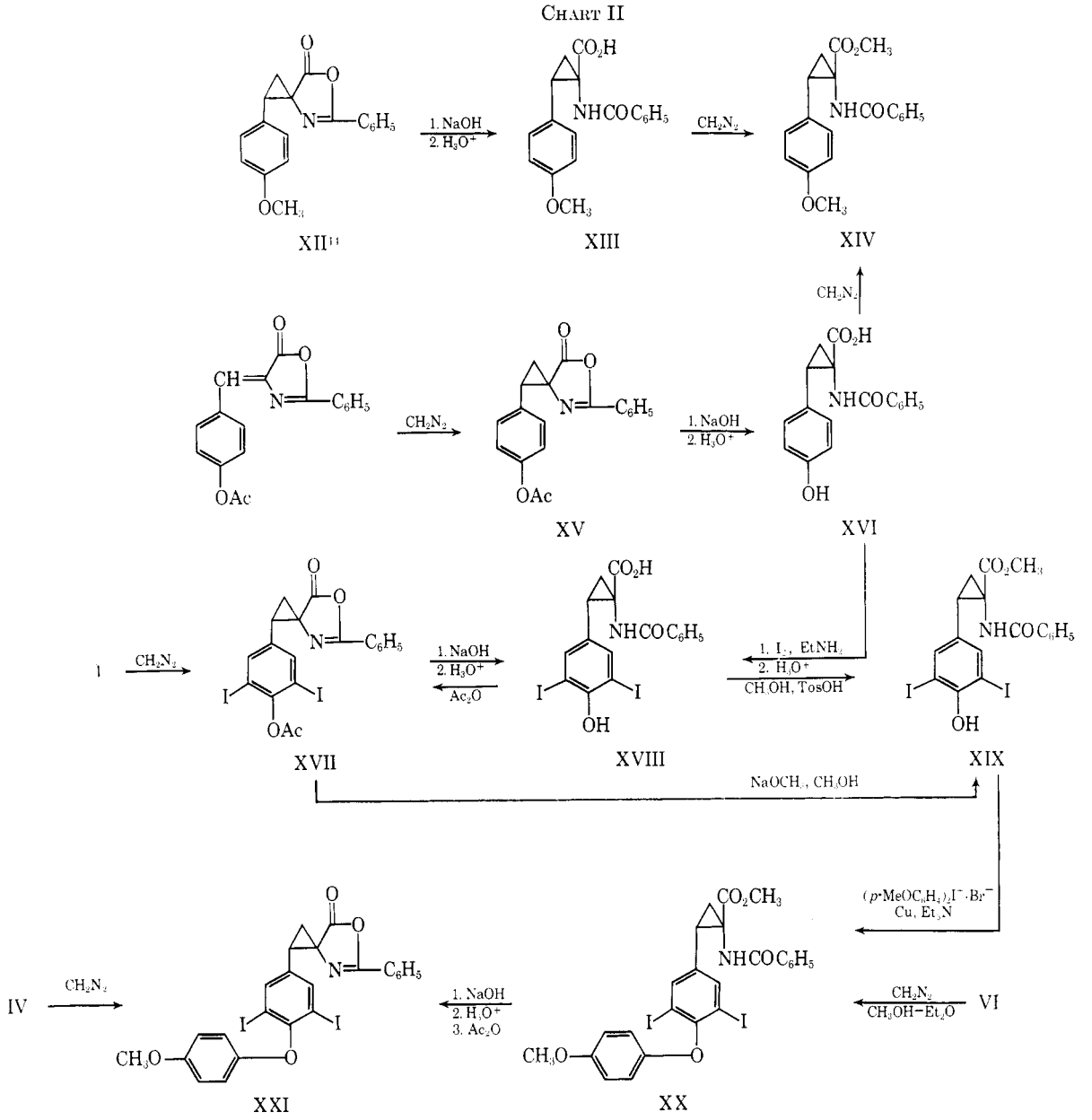
The spirooxazolone XII has been described by Awad, *et al.*,[14] without comments on its stereochemistry. It was prepared by the addition of diazomethane to the corresponding unsaturated oxazolone. This spirooxazolone XII was different from the isomeric compound XI which in turn was prepared by us from 1-amino-*cis*-2-(4-methoxyphenyl)cyclopropanecarboxylic acid (IX). The configuration of IX follows from its formation from diethyl 2-(4-methoxyphenyl)-1,1-cyclopropanedicarboxylate.[12] Partial saponification of this ester removed only the *trans* alkoxyl group, and the

(19) C. P. Leblond and B. Grad, *J. Pharmacol. Exptl. Therap.*, **94**, 125 (1948); *cf.* E. Abderhalden and E. Wertheimer, *Z. Ges. Exptl. Med.*, **63**, 557 (1928).

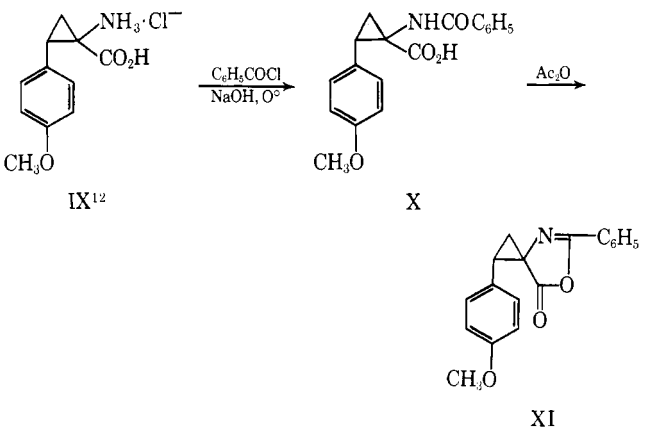
(20) R. Filler, *Advan. Heterocyclic Chem.*, **4**, 95 (1965).

(21) A. Mustafa, W. Asker, A. H. Harhash, and A. M. Fleifel, *Tetrahedron*, **21**, 2215 (1965).

CHART II



cis-carbethoxy-*trans*-carboxylic acid thus obtained was converted to IX by Curtius degradation of the free carboxyl.[22] Retention of configuration in the benzoylation[23] of IX and in the ring closure[24,25] of X is based on

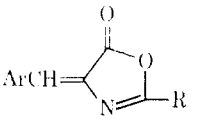


pertinent analogies in the literature. Therefore, the oxazolone carbonyl of XII is *trans* to the methoxyphenyl group.

The relationship of XII to VI was established by the reactions shown in Chart II. The spirooxazolone XII was hydrolyzed to the benzamido acid XIII which differed from the isomer X. Esterification of XIII furnished the ester XIV which was also obtained from the phenolic benzamido acid XVI, separately synthesized from the *p*-acetoxy azlactone XV. Iodination of XVI led to the cyclopropylog of 3,5-diiodo-*N*-benzoyltyrosine (XVIII) which in turn was also synthesized from I as shown in Chart II. The methyl ester (XIX) of XVIII was coupled with di(*p*-anisyl)-iodonium bromide to yield the thyronine analog XX. This compound was also formed from VI with diazomethane in methanol–ether.

(22) For analogous cases see ref 5 and 11.
(23) R. E. Steiger, *J. Org. Chem.*, **9**, 396 (1944).
(24) R. E. Buckles, R. Filler, and L. Hilfman, *ibid.*, **17**, 233 (1952).
(25) N. K. Kochetkov, E. I. Budovskii, R. M. Khomutov, M. Ya. Karpeiskii, and E. S. Severin, *Zh. Obshch. Khim.*, **30**, 2573 (1960); *J. Gen. Chem. USSR*, **30**, 2557 (1960).

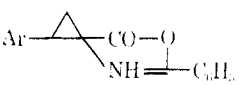
TABLE I



Ar	R	Yield (%), method	Mp, °C	Compn	Calcd, % C	Calcd, % H	Found, % C	Found, % H
4-AcO-3,5-$I_2C_6H_2$	C_6H_5	80, A	242–242.5[a]	$C_{18}H_{11}I_2NO_4$	38.67	1.98	38.40	1.89
4-$C_6H_5CH_2O$-3,5-$I_2C_6H_2$	C_6H_5	72, A	211–212[b]	$C_{23}H_{15}I_2NO_3$	45.50	2.49	45.57	2.65
4-(4-$AcOC_6H_4O$)-3,5-$I_2C_6H_2$	C_6H_5	69, B	233–234[c]	$C_{24}H_{15}I_2NO_5$	44.27	2.32	44.38	2.50
4-(4-$AcOC_6H_4O$)-3,5-$I_2C_6H_2$	CH_3	44, B	240–242[c]	$C_{19}H_{13}I_2NO_5$	38.74	2.22	38.55	2.43
4-(4-$CH_3OC_6H_4O$)-3,5-$I_2C_6H_2$	CH_3	63, B	219–221	$C_{18}H_{13}I_2NO_4$	38.53	2.34	38.39	2.50

[a] From EtOAc–hexane. [b] From CCl_4. [c] From AcOH.

TABLE II



Ar	Yield (%), method	Mp, °C	Compn	Calcd, % C	Calcd, % H	Found, % C	Found, % H	Infrared absorption, oxazolone C=O, cm^{-1}
4-$CH_3OC_6H_4$ (XI)[a]	53, D	137–138[b]	$C_{16}H_{15}NO_3$	73.70	5.15	73.90	5.24	1805
4-$AcOC_6H_4$ (XV)	38, C	147.5–148[b]	$C_{19}H_{15}NO_4$	71.02	4.36	71.20	4.51	1820
4-AcO-3,5-$I_2C_6H_2$	50, C; 90, D	228.5–229[b]	$C_{19}H_{13}I_2NO_4$	39.82	2.29	40.01	2.40	1820
4-$C_6H_5CH_2O$-3,5-$I_2C_6H_2$	55, C	199.5–200[c]	$C_{24}H_{17}I_2NO_3$	46.40	2.76	46.23	2.58	1820
4-(4-$AcOC_6H_4O$)-3,5-$I_2C_6H_2$	29, C	213–215[d,e]	$C_{25}H_{17}I_2NO_5$	45.14	2.58	45.30	2.78	1820
4-(4-$CH_3OC_6H_4O$)-3,5-$I_2C_6H_2$	32, C; 46, D	224–225[e]	$C_{24}H_{17}I_2NO_4$	45.24	2.69	45.35	2.87	1815

[a] Obtained from IX *via* X; the 2-(4-methoxyphenyl) substituent is therefore *cis* to oxazolone C=O. [b] From EtOAc–hexane. [c] From EtOAc. [d] From AcOH. [e] When crystallized from EtOAc, 1 mole of EtOAc of crystallization was found. *Anal.* Calcd for $C_{25}H_{17}I_2NO_5 \cdot C_4H_8O_2$: C, 46.24; H, 3.35. Found: C, 46.18, 46.35; H, 3.40, 3.61.

Experimental Section[26]

General Method for Unsaturated Azlactones. A. From Aldehydes.—A mixture of equimolar quantities of the aromatic aldehyde, hippuric acid, and anhydrous sodium acetate, and enough acetic anhydride to cover the solids was heated in a dish on a steam bath for 1 hr. The cooled residue was ground with an amount of water corresponding to that of the acetic anhydride used and filtered, and the crystals were washed with water until free from acetic acid. The air-dried crystals were recrystallized (see Table I). For infrared spectra, see text.

B. From Methyl α-Acylamino-β-arylacrylates.—A methanolic solution of the ester was hydrolyzed with 1.5 moles of KOH (20% in H_2O) at reflux for 12 hr. The solution was poured into 2 vol. of ice–water and acidified to pH 1, and the crude solid acylamino acid was filtered off. The material was dried (vacuum, P_2O_5) and then heated on a steam bath with two parts of acetic anhydride for 0.5 hr. The cooled residue was ground with four parts of water and filtered. The crystals were washed with water, dried over P_2O_5, and recrystallized from a suitable solvent.

General Procedure for 1-Aryl-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-ones. Method C. From Unsaturated Oxazolones (Table II).—A dry solution of about 0.233 mole of diazomethane (from 48 g of N-methyl-N-nitrosourea) in 2.5 l. of ether[27] was added with stirring to a solution of 0.04–0.05 mole of the unsaturated oxazolone in enough $CHCl_3$[28] to dissolve the compound,[27] and the mixture was stirred at 26° for 12 hr. It was filtered to remove any product and/or polymethylenes which were washed with dry ether. Any filtered product was crystallized from a suitable solvent; from the filtrate the spirooxazolones were recovered by concentration to 100–200 ml and cooling. The precipitated products were filtered off and washed with ether.

Method D. From 1-Acylamino-2-arylcyclopropanecarboxylic Acids.—The acid (0.5 g) was heated on a steam bath with 1 ml of acetic anhydride for 0.5 hr, the mixture was cooled, and the azlactone was filtered off and washed with ether. If the azlactone did not crystallize, 2 ml of ether was added, and the mixture cooled to 4° overnight until crystallization had taken place.

If the methyl ester of the acylamino acid was available, the methanolic solution of the ester was hydrolyzed with 1.5 moles of 20% aqueous KOH at reflux for 12 hr. The solution was poured into 2 vol. of ice–water and acidified to pH 1, and the crude acylamino acid was filtered off. The dried (P_2O_5, vacuum) material was used directly as above.

Principal Synthetic Path to 1-Amino-2-[3,5-diiodo-4-(4-hydroxyphenoxy)phenyl]cyclopropanecarboxylic Acid. 4-(4-Acetoxy-3,5-diiodobenzylidene)-2-phenyl-2-oxazolin-5-one (I) was prepared from 3,5-diiodo-4-hydroxybenzaldehyde by method A;[29] infrared, 1790 ($\nu_{C=O}$ oxazolone), 1760 ($\nu_{C=O}$ acetoxy), 1650 cm^{-1} ($\nu_{C=N}$ oxazolone); ultraviolet (CH_3CN), 384 mμ (ε 26,900), 364 (39,200), 350 (sh) (32,000), 257 (18,900), 241 (19,200).

Methyl α-Benzamido-β-(3,5-diiodo-4-hydroxyphenyl)acrylate (II).—A mixture of 376 g (0.673 mole) of I, 54 g (1.0 mole) of sodium methoxide, and 1500 ml of absolute methanol was stirred under reflux for 2 hr, cooled, poured into ice–water, and filtered. The clear filtrate was acidified to pH 3 with concentrated HCl and filtered. The crystals were washed with H_2O, dried, and recrystallized from acetic acid to yield 225 g (62%) of II, mp 215.5–216.5°. An analytical sample recrystallized from acetic acid had mp 218–220°; infrared, 3400 (ν_{OH} phenol), 3240 (ν_{NH} amide), 1715 ($\nu_{C=O}$ ester), and 1655 cm^{-1} ($\nu_{C=O}$ amide).

Anal. Calcd for $C_{17}H_{13}I_2NO_4$: C, 37.19; H, 2.39. Found: C, 37.07; H, 2.32.

Methyl α-Benzamido-β-[3,5-diiodo-4-(4-methoxyphenoxy)-phenyl]acrylate (III).—A mixture of 224 g (0.408 mole) of II, 345 g (0.82 mole) of dianisyliodonium bromide,[30] 50 ml of triethylamine, 5 g of copper powder, and 2 l. of absolute methanol was stirred for 24 hr at 23°. The crystals were removed by

(26) Melting points were taken in a capillary melting point apparatus preheated to about 15° below the reported values and are corrected. Infrared spectra were determined on a Perkin-Elmer Model 337 spectrophotometer as KBr pellets. Ultraviolet spectra were recorded on a Beckman DU spectrophotometer. Nmr spectra were run with a Varian A-60 spectrometer and are reported as parts per million units downfield from $(CH_3)_4Si$ used as an internal standard unless indicated otherwise. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

(27) In the case of the 4-acetoxy- and 4-benzyloxy-3,5-diiodophenyl derivatives, a suspension of the azlactone in 500 ml of $CHCl_3$ was used.

(28) In the case of 4-[4-(4-acetoxyphenoxy)-3,5-diiodobenzylidene]-2-phenyl-2-oxazolin-5-one, dioxane was used instead of $CHCl_3$ to obtain a homogeneous solution.

(29) T. Matsuura and H. J. Cahnmann, *J. Am. Chem. Soc.*, **81**, 871 (1959).

(30) H. Ziegler and C. Marr, *J. Org. Chem.*, **27**, 3335 (1962).

filtration, dissolved in boiling 2-butanone, and filtered, and the filtrate was cooled to yield 150 g (56%) of colorless III, mp 214–216°. An analytical sample, recrystallized from 2-butanone–ethyl acetate had mp 221–223°; infrared, 3230 (ν_{NH} amide), 1715 ($\nu_{C=O}$ ester), and 1650 cm^{-1} ($\nu_{C=O}$ amide).

Anal. Calcd for $C_{24}H_{19}I_2NO_5$: C, 43.99; H, 2.92. Found: C, 43.95; H, 2.74.

4-[3,5-Diiodo-4-(4-methoxyphenoxy)benzylidene]-2-phenyl-2-oxazolin-5-one (IV).—This compound, previously described by Harington and Barger,[15] was obtained from III by the general method B in 78% yield, mp 217–219° (lit.[15] mp 211°). A comparison with a sample prepared by the literature method[15] (our method A) (mp 214.5–215.5°) showed no mixture melting point depression; the infrared spectra of the two samples were identical.

4-[4-(Acetoxyphenoxy)-3,5-diiodobenzylidene]-2-phenyl-2-oxazolin-5-one (V).—A suspension of 47 g (0.0755 mole) of IV in 500 ml of anhydrous CH_2Cl_2 was cooled to −70° and treated with a solution of 100 g (0.4 mole) of boron tribromide in 100 ml of anhydrous CH_2Cl_2. After stirring at −70° for 0.5 hr, the mixture was allowed to warm to 25° (2 hr). During this period the suspended solid dissolved. The clear dark solution was poured into 2 l. of ice–water; the precipitated yellow solid was filtered, washed with water, and dried. The layers of the filtrate were separated, the water layer was extracted with 100-ml portions of CH_2Cl_2, and the combined extracts were washed (H_2O), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residual yellow solid was combined with the dried filtered material (above), 150 ml of acetic anhydride was cautiously added, and the mixture was heated on a steam bath for 0.5 hr. The cooled residue was ground with H_2O and filtered, and the crystals were washed with H_2O and dried. The crude product was then heated with 150 ml of boiling acetic acid for 5 min, cooled, and filtered, and the yellow crystals were washed well with hexane and air dried. For physical and analytical data, see Table I; infrared, 1790 ($\nu_{C=O}$ oxazolone), 1760 ($\nu_{C=O}$ acetoxy), and 1660 cm^{-1} ($\nu_{C=N}$ oxazolone); ultraviolet (CH_3CN), 386 mμ (ε 30,700), 368 (42,700), 355 (sh) (34,900), 258 (19,300), and 242 (20,500).

1-[4-(4-Acetoxyphenoxy)-3,5-diiodophenyl]-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-one (VI) was prepared from V by the general method C; infrared, 1820 ($\nu_{C=O}$ oxazolone), 1760 ($\nu_{C=O}$ acetoxy), 1635 cm^{-1} ($\nu_{C=N}$ oxazolone); ultraviolet (CH_3CN), 263 mμ (ε 23,600).

1-Amino-*trans*-2-[4-(4-hydroxyphenoxy)-3,5-diiodophenyl]-cyclopropanecarboxylic Acid Hydrochloride (VII).—A mixture of 1.95 g (0.00293 mole) of VI, 40 ml of concentrated HCl, 30 ml of H_2O, and 30 ml of acetic acid was stirred under reflux while a slow stream of nitrogen was passed through the reaction flask. The initially clear solution became cloudy after 11 hr and then cleared again after about 23 hr. After 36 hr the clear, yellow solution was distilled at atmospheric pressure until 25–30 ml of distillate had been collected. Upon cooling to room temperature 0.28 g of benzoic acid (as shown by its infrared spectrum and a mixture melting point with an authentic sample) separated and this was removed by filtration. Extraction of the filtrate with three 50-ml portions of ether yielded, on evaporation, an additional 0.05 g of benzoic acid; total yield 0.33 g (92%).

The clear, aqueous layer became cloudy upon standing at room temperature for a few minutes; after standing at −17° for 48 hr, the small, faintly pink crystals were filtered and air dried to give 0.70 g of VII. Concentration of the filtrate *in vacuo* to 10 ml yielded an additional 0.21 g of product; total yield 0.91 g (54%). An analytical sample, prepared by recrystallization (Darco) from 6 *N* HCl containing a few milliliters of acetic acid, had mp 185–187° after prior sintering at 165°.

Anal. Calcd for $C_{16}H_{14}ClI_2NO_4 \cdot H_2O$: C, 32.49; H, 2.73. Found: C, 32.80, 32.70; H, 2.75, 2.80.

If the hydrolysis of VI was allowed to proceed for only 12 hr and the product was isolated as above, only a small quantity of benzoic acid was obtained and the major product was 1-benzamido-*trans*-2-[4-(4-hydroxyphenoxy)-3,5-diiodophenyl]cyclopropanecarboxylic acid, which after recrystallization from ethyl acetate–hexane, appeared as colorless crystals: mp 216–217°; infrared, 3380 (ν_{OH} phenol), 3280 (ν_{NH} amide), 1700 ($\nu_{C=O}$ acid), and 1640 cm^{-1} ($\nu_{C=O}$ amide).

Anal. Calcd for $C_{23}H_{17}I_2NO_5$: C, 43.08; H, 2.67. Found: C, 42.91; H, 2.82.

1-Amino-*trans*-2-[4-(3,5-dibromo-4-hydroxyphenoxy)-3,5-diiodophenyl]cyclopropanecarboxylic Acid (VIII).—A solution (2.45 ml, 0.93 mole) of bromine in glacial acetic acid (6.1 g/100 ml) was added dropwise to a solution of 0.27 g (0.457 mole) of VII in 5 ml of the same solvent. The orange-red solution was stirred at 28° for 20 hr, cooled, neutralized with 40% aqueous methylamine to pH 4–5, diluted with 10 ml of water, and allowed to stand at 4° for 6 hr. A nearly colorless precipitate (0.32 g) formed; it was filtered and washed with water. The crude material was dissolved in a mixture of 5 ml of acetic acid, 2 drops of 37% HCl, 1 ml of methanol, and 3 ml of water. The solution was treated with Darco and then neutralized (pH 4–5) with 20% aqueous methylamine. Repetition of this procedure yielded 0.12 g of colorless material which was dried (P_2O_5, vacuum), mp 167–170°. This product contained a small amount of a second component (tlc on silica gel–aqueous 1-propanol, 70% v/v) which could not be removed by further reprecipitation.

Anal. Calcd for $C_{16}H_{11}Br_2I_2NO_4$: C, 27.66; H, 1.60; N, 2.02. Found: C, 27.48, 27.69; H, 1.89, 2.01; N, 1.69.

1-Benzamido-*cis*-2-(4-methoxyphenyl)cyclopropanecarboxylic acid (X) was prepared from IX by the general method of Steiger[23] for the benzoylation of amino acids. The crude compound, mp 197–199°, was obtained in 50% yield. Recrystallization from ethanol–water gave colorless material: mp 198–199.5°; infrared, 3360 (ν_{NH} NHCO), 1700 ($\nu_{C=O}$ CO_2H), 1635 cm^{-1} ($\nu_{C=O}$ NHCO).

Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50. Found: C, 69.23; H, 5.56.

***cis*-1-(4-Methoxyphenyl)-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-one (XI)** was prepared from X by the general method D, mp 137–138°; infrared, 1805 ($\nu_{C=O}$ oxazolone), 1640 cm^{-1} ($\nu_{C=N}$ oxazolone). A mixture melting point with XII[14,31] (mp 124–125°) (lit.[14,31] 124°) was 110–134°.

1-Benzamido-*trans*-2-(4-methoxyphenyl)cyclopropanecarboxylic Acid (XIII).—A solution of 3.21 g (0.011 mole) of XII in 25 ml of 2% aqueous NaOH and 25 ml of acetone was refluxed for 30 min, poured into ice–water, acidified to pH 1, and cooled to 4°. The colorless crystals which separated were filtered off, washed with water, and dried (P_2O_5, vacuum); yield 3.26 g (96%), mp 225–230°. Recrystallization from ethanol–water gave 2.9 g (85%) of pure material: mp 235–237°; infrared, 3290 (ν_{NH} NHCO), 1690 ($\nu_{C=O}$ CO_2H), 1660 cm^{-1} ($\nu_{C=O}$ NHCO).

Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.56. Found: C, 69.18; H, 5.66.

When XIII was treated with a slight excess of diazomethane in ether containing 1% of methanol, **methyl 1-benzamido-*trans*-2-(4-methoxyphenyl)cyclopropanecarboxylate (XIV)** was formed, near quantitatively, mp 174.5–175° after recrystallization from methanol–water; infrared, 3340 (ν_{NH} NHCO), 1740 ($\nu_{C=O}$ CO_2CH_3), 1645 cm^{-1} ($\nu_{C=O}$ NHCO); nmr ($CDCl_3$), δ 7.14 (9 H, multiplet, aromatic *H*), 6.28 (1 H, singlet, CON*H*C_6H_5), 3.77 (3 H, singlet, OC*H*$_3$), 3.72 (3 H, singlet, OC*H*$_3$), 3.00 (1 H, multiplet, cyclopropane C*H*), 2.21 (1 H, multiplet, cyclopropane C*H*), 1.78 (1 H, multiplet, cyclopropane C*H*).

Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89. Found: C, 69.98; H, 5.67.

1-(4-Acetoxyphenyl)-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-one (XV) was prepared from 4-(4-acetoxybenzylidene)-2-phenyl-2-oxazolin-5-one[32] by method C; infrared, 1820 ($\nu_{C=O}$ oxazolone) 1755 ($\nu_{C=O}$ acetoxy), 1635 cm^{-1} ($\nu_{C=N}$ oxazolone); nmr (pyridine), δ 3.33 (1 H, multiplet, cyclopropane-*H*), 2.17 (5 H: singlet superimposed on multiplet, OCOC*H*$_3$, cyclopropane C*H*$_2$).

1-Benzamido-*trans*-2-(4-hydroxyphenyl)cyclopropanecarboxylic Acid (XVI).—A mixture of 11.1 g (0.034 mole) of XV in 100 ml of 5% aqueous NaOH and 100 ml of acetone was stirred under reflux for 2.5 hr. The cooled solution was poured into ice–water and acidified to pH 1, and the mixture was allowed to stand at 4° for 2 hr. The buff-colored crystals which separated were filtered off, washed with H_2O, and dried over P_2O_5 to give 6.7 g (65%) of XVI, mp 224–226°. An analytical sample prepared by recrystallization from methanol had mp 231–232°; infrared, 1705 ($\nu_{C=O}$ COOH), 1655 cm^{-1} ($\nu_{C=O}$ NHCO).

Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09. Found: C, 68.86; H, 5.18.

When XVI was treated with excess CH_2N_2 in ether containing 16% of methanol for 30 hr at 25°, colorless crystals separated out, mp 168–170°. After recrystallization from methanol–water,

(31) We found for XII infrared 1810 ($\nu_{C=O}$ oxazolone), 1635 cm^{-1} ($\nu_{C=N}$ oxazolone).

(32) E. Erlenmeyer and W. Stadlin, *Ann.*, **337**, 286 (1904).

the melting point was 170–173°, undepressed on admixture of XIV. The infrared spectra of the two materials were superimposable.

1-Benzamido-*trans*-2-(3,5-diiodo-4-hydroxyphenyl)cyclopropanecarboxylic Acid (XVIII).—To a solution of 2.97 g (0.01 mole) of XVI in 50 ml of 33% aqueous ethylamine was added slowly, with stirring, over 1 hr, 5.34 g (0.021 mole) of iodine in 125 ml of 10% aqueous KI. Stirring was continued for 1 hr at 25°. The solution was poured into ice–water, neutralized to pH 1, and cooled. The nearly colorless crystals were removed by filtration, air dried, and recrystallized from acetic acid; yield 3.0 g (55%), mp 210–214°. This material proved to be identical with XVIII prepared from XVII (see below) as shown by a comparison of their infrared spectra and mixture melting point which showed no depression.

1-(4-Acetoxy-3,5-diiodophenyl)-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-one (XVII) was prepared from I by general procedure C; infrared, 1820 ($\nu_{C=O}$ oxazolone), 1765 ($\nu_{C=O}$ acetoxy), 1635 cm^{-1} ($\nu_{C=N}$ oxazolone); ultraviolet (CH_3CN), 263 mμ (ε 19,700).

A mixture of 17.8 g (0.031 mole) of XVII, 200 ml of 2% aqueous NaOH, and 200 ml of acetone was stirred under reflux for 4.5 hr and the acetone was removed under reduced pressure. The residue was poured into ice–water, acidified to pH 1, and cooled at 4° for 2 hr. The buff-colored crystals which separated were filtered, washed with H_2O, and dried over P_2O_5 to yield 14.0 g (82%) of XVIII, mp 214–215°. An analytical sample prepared by recrystallization from acetic acid had mp 215.5–216.5°; infrared, 3450 (ν_{OH} phenol), 3280 (ν_{NH} NHCO), 1700 ($\nu_{C=O}$ COOH), 1645 cm^{-1} ($\nu_{C=O}$ NHCO).

Anal. Calcd for $C_{17}H_{13}I_2NO_4$: C, 37.19; H, 2.39. Found: C, 37.09; H, 2.50.

This material was identical with that obtained by iodination of XVI (mixture melting point and infrared spectra).

Methyl 1-Benzamido-*trans*-2-(3,5-diiodo-4-hydroxyphenyl)-cyclopropanecarboxylate (XIX). A. From XVIII.—A mixture of XVIII (10 g, 0.018 mole), 50 ml of absolute methanol, 100 ml of 1,2-dichloroethane, and 0.5 g of *p*-toluenesulfonic acid was refluxed for 13 hr. The cooled reaction mixture was washed with H_2O, whereupon the product crystallized from the organic layer. The colorless crystals were filtered and recrystallized from methanol to yield 6.4 g (62%) of XIX, mp 195–197°. An analytical sample, recrystallized from methanol, had mp 199–200°; infrared, 3450 (ν_{OH} phenol), 3280 (ν_{NH}NHCO), 1725 ($\nu_{C=O}$ CO_2CH_3), 1645 cm^{-1} ($\nu_{C=O}$ NHCO).

Anal. Calcd for $C_{18}H_{15}I_2NO_4$: C, 38.39; H, 2.68. Found: C, 38.53; H, 2.84.

B. From XVII.—A mixture of XVII (16.4 g, 0.029 mole) and 3.24 g (0.06 mole) of sodium methoxide in 200 ml of absolute methanol was stirred under reflux for 3 hr. The solution was poured into ice–water and filtered, the filtrate was acidified to pH 3, and the mixture was cooled at 4° for 2 hr. The colorless crystals were filtered, washed with H_2O, and dried (vacuum, P_2O_5) to yield 15 g (93%) of XIX, mp 187–189°. The infrared spectrum of this material was identical with that of the product from method A above. Recrystallization from methanol gave colorless crystals, mp 197–199°.

Methyl 1-Benzamido-*trans*-2-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]cyclopropanecarboxylate (XX). A. From XIX.—A mixture of 15 g (0.026 mole) of XIX, 23.2 g (0.055 mole) of dianisyliodonium bromide,[30] 10 ml of triethylamine, 5 g of copper powder, and 200 ml of absolute methanol was stirred at 25° for 24 hr. It was filtered, the brownish crystals were dissolved in boiling methanol and filtered, and the filtrate was cooled to give 6.45 g (37%) of colorless XX, mp 185–187°. An analytical sample prepared by recrystallization from methanol had mp 186.5–187.5°; infrared, 3320 (ν_{NH} NHCO), 1735 ($\nu_{C=O}$ CO_2CH_3), 1645 ($\nu_{C=O}$ NHCO); nmr ($CDCl_3$), δ 7.78 (2 H, singlet, 2,6 protons of iodine-substituted ring), 7.48 (5 H, multiplet, phenyl protons of benzamido group), 6.72 (4 H, multiplet, protons of 1,4-disubstituted ring), 6.43 (1 H, broad singlet, $NHCOC_6H_5$), 3.75 (6 H, singlet, OCH_3, methyl ether and methyl ester), 3.08 (1 H, multiplet, cyclopropane CH), 2.03 (2 H, multiplet, cyclopropane CH_2); nmr (pyridine), δ 3.72 and 3.63 (3 H, singlet, OCH_3, methyl ether or methyl ester), 3.31 (1 H, multiplet, cyclopropane CH), 2.23 (2 H, multiplet, cyclopropane CH_2).

Anal. Calcd for $C_{25}H_{21}I_2NO_5$: C, 44.87; H, 3.16. Found: C, 44.69; H, 3.16.

B. From VI.—Treatment of VI with excess diazomethane in ether containing 16% methanol for 20 hr at 25° followed by evaporation of the solvent under reduced pressure furnished a residue which was crystallized from methanol–water. The resulting colorless crystals had mp 175–183°, undepressed (181–186°) by admixture of a sample obtained by method A from XIX. The infrared spectra of the two materials, and their respective nmr spectra in $CDCl_3$ and in pyridine, were identical.

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The Synthesis and Biological Evaluation of 16β-Amino-17α,20-dihydroxypregnanes

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The addition of primary and cyclic secondary amines to 16,17α-epoxy-20-hydroxypregnenes gave a series of 16β-amino-17α,20-dihydroxypregnenes. The amines were broadly screened and showed some activity as antihypertensive, antibacterial, antiprotozoal, and analgesic agents. The method of molecular rotation differences was shown to be applicable to the determination of configuration at C-20 in the 16,17α-epoxy-20-hydroxypregnane series.

The search for steroids with increased biological utility has led to the synthesis of such a profusion of compounds that hardly a position on the nucleus has resisted the introduction of a variety of new substituents. Molecular manipulation at the 16-carbon atom[1] has shown this to be one of the more profitable sites on which to operate. The introduction of a 16α-hydroxyl or a 16α-methyl group has been the most successful alteration to date. In the evolution of altered steroids the addition of nitrogen substituents has been a relatively recent development spurred by the success of the ring A pyrazoles.[2] Of the naturally occurring steroids the solanum alkaloids[3] and the apocynaceae[4] possess a C-16 nitrogen function and have been ex-

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