Cooling and filtration gave a solid which was washed with $H_2O$ and dried: 76.1 g; mp 135–147°. Recrystallization from EtOAc left 56.2 g (75%) of 11, mp 153–154.5° (lit.[12] mp 154°).

**1,4-Bis(*p*-anisyl)butane-1,4-diol (12).** Diketone 11 (96.5 g) was reduced with $NaBH_4$ by the method reported[11] for reduction of 1,2-dibenzoylethane. The product was obtained as a mixture of meso and *dl* forms: 93.9 g (96%); mp 104–122°. The diol of this quality was suitable for use in the next step. On one occasion, a single isomeric form of the diol was obtained by repeated recrystallization of the crude product from EtOAc: mp 138.5–139.5° *Anal.* ($C_{18}H_{22}O_4$) C, H.

**1,4-Bis(*p*-anisyl)-1,4-dichlorobutane (13).** A solution of diol 12 (31.7 g, 0.11 mol) in 800 ml of HOAc–dioxane (4:1) was stirred at 5–10° while a stream of dry HCl gas was bubbled into the liquid at a moderate rate for 2 hr. Heptane (800 ml) was added, and the precipitated solid was collected on a filter and washed with heptane: 32.2 g (90%). Recrystallization (acetone) gave a refined sample. *Anal.* ($C_{18}H_{20}O_2Cl_2$) C, H, Cl. This material decomposed at 124.5°, evolving gas and only partially melting. Complete melting finally occurred at 219°, suggesting thermal conversion of the dichloride to 1,4-bis(*p*-anisyl)-1,4-butadiene (lit.[18] mp 229–231°).

**1,2-Bis(*p*-anisyl)cyclobutene (14).** Cyclization of 13 was done with $NaNH_2$ by the procedure reported[11] for the phenyl analog. Work-up gave a dark oil which was chromatographed on alumina (benzene). Product-containing fractions (located by tlc) were evaporated to an oil which crystallized from cold MeOH. In a run using 27.2 g of 13 there was obtained 13.5 g (64%), mp 85.5–87°. Recrystallization (EtOH) gave 12.1 g (57%) of 14, mp 88.5–89.5°. *Anal.* ($C_{18}H_{18}O_2$) C, H.

***cis*-1,2-Bis(*p*-anisyl)cyclobutane (2b).** Cyclobutene 14 (8.15 g, 0.031 mol) was hydrogenated over 0.2 g of 10% Pd/C in 100 ml of dioxane. Filtration and evaporation of the filtrate left an oil which was dissolved in MeOH. Cooling in a Dry Ice–MeOH bath produced **2b**: 7.20 g (88%); mp 38–39.5°. Recrystallization (MeOH) gave 5.75 g (70%): mp 40.5–41.5°; nmr (DMSO-$d_6$) $\delta$ 3.82 (m, 2, benzylic H). *Anal.* ($C_{18}H_{20}O_2$) C, H.

***trans*-1,2-Bis(*p*-anisyl)cyclobutane (2c).** Isomerization of 2b was effected by KO-*t*-Bu in DMSO as described[11] for the phenyl analog. From 13.68 g of **2b** there was obtained 11.10 g of oily **2c**. Chromatography on alumina (benzene) followed by distillation gave 10.25 g (75%): bp 156–175° (0.1 mm); $n^{25}D$ 1.5783; nmr (DMSO-$d_6$) $\delta$ 3.31 (m, 3, benzylic H). *Anal.* ($C_{18}H_{20}O_2$) C, H.

**Acknowledgments.** The authors are thankful to Charles M. Combs, Clarence I. Kennedy, and John G. Schmidt for analytical data and to John P. Braselton, Ray F. Pritchett, and Clelia Millstead for assistance with the biological testing.

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# Synthesis, Estrogenic Activity, and Electrophilic Reactivity of an *N*-Acetoxy-*N*-acetamido Analog of Diethylstilbestrol

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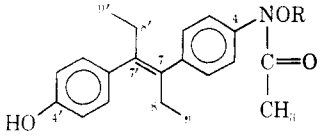
*Received October 29, 1973*

*N*-Hydroxy- and *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*trans*-*N*-4-stilbenylacetamide were synthesized for study of their carcinogenic and estrogenic activities. These compounds are analogs of diethylstilbestrol and also of the potent carcinogens *N*-hydroxy- and *N*-acetoxy-*N*-4-*trans*-stilbenylacetamide. The hydroxamic acid and the *N*-acetoxyamide exhibited 1/600 and 1/400, respectively, of the estrogenic activity of diethylstilbestrol. The electrophilic reactivity at neutrality of the *N*-acetoxyamide toward methionine was of the same order of magnitude as that of *N*-acetoxy-*N*-4-*trans*-stilbenylacetamide and about 1/10 that of *N*-acetoxy-*N*-2-fluorenylacetamide.

The potent estrogen diethylstilbestrol (4,4′-dihydroxy-7,7′-diethyl-*trans*-stilbene), which is carcinogenic in estrogen target organs of a number of species including the human,[1-3] and the potent carcinogens *N*-hydroxy- and *N*-acetoxy-*N*-4-*trans*-stilbenylacetamide[4,5] share some structural features. The objective of the present work was to design a new carcinogen with specificity for the organ and subcellular targets of the estrogens through combination of the essential structural features of the estrogens and the *trans*-stilbenylacetamide carcinogens. This paper describes the synthesis of *N*-hydroxy- (**10**) and *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide (**11**). These compounds are estrogenic although much less active than diethylstilbestrol. The *N*-acetoxyamide shows similar electrophilic reactivity to that of *N*-acetoxy-*N*-4-*trans*-stilbenylacetamide, a property which appears to be essential for an ultimate carcinogen.[6,7] The compounds are being assayed for carcinogenic activity.




10. R = H
11. R = C(=O)$CH_3$

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## Experimental Section

Corrected melting points were determined from inflections in rapid time-temperature curves obtained with the Accumelt apparatus (American Instruments Co., Silver Springs, Md.). Elemental analyses were made by Huffman Laboratories, Inc., Wheatridge, Colo. Where analyses are indicated only by symbols of the elements, the results obtained for those elements were within ±0.4% of the theoretical values. Mass spectra were determined with a Varian CH-7 mass spectrometer (Varian-MAT, Bremen, Germany); perfluorokerosene (Penninsular ChemResearch, Inc., Gainesville, Fla.) was used as an internal standard for calibration of the mass units.

Ir spectra were recorded with a Beckman IR-10 spectrophotometer. Uv absorbance was determined with a Beckman DB spectrophotometer, equipped with a Sargent SR recorder; 60-MHz nmr spectra were measured with spectrometer R12 (Perkin-Elmer). Ir values are expressed in reciprocal centimeters and chemical shifts in $\delta$ units with TMS as internal reference. Aluminum sheets precoated with silica gel F-254 (fast running, E. Merck, Germany) were used for analytical tlc. Plates for preparative tlc were prepared from silica gel PF 254 (Merck). All chromatograms were developed in an equilibrated atmosphere. The solvent mixtures were prepared according to volume measurements. The products were located by visualization under uv light (254 nm) and/or by spraying with vanillin-sulfuric acid reagent (VS) and subsequent heating of the plates at 110° for about 10 min.[8]

**4-Nitro-4′-methoxydeoxybenzoin**[9] (1). The synthesis of Jenkins and Wilkinson[9] yielded 1 as yellow needles (68%): mp 110–112° (lit.[9] 110–111°); $R_f$ 0.22 ($CH_2Cl_2$–*n*-hexane, 1:1); (VS) violet; ir (KBr) 1680 (C=O), 1520, and 1350 ($NO_2$).

**4-Nitro-4′-methoxy-α-ethyldeoxybenzoin**[10] (2). 1 was ethylated as described by Rubin and Wishinsky.[10] Since no purification of the reaction product was achieved by fractional distillation,[10] the residue (9 g in $CH_2Cl_2$) was chromatographed on a silica gel column (3 × 58 cm) (Merck, 0.02–0.2 mm mesh, deactivated with 13% $H_2O$). The column was sequentially eluted with 1000-ml aliquots of mixtures of *n*-hexane–$CH_2Cl_2$ (6:1, 5:1, 3:2). The product, which emerged as the third yellow band, was a yellow oil (overall yield, 53%) after the solvent had been removed under vacuum: $R_f$ 0.38 (*n*-hexane–$CH_2Cl_2$, 1:1); (VS) black; ir ($CHCl_3$) 1680 (C=O), 1520, 1350 ($NO_2$); nmr ($CDCl_3$) 8.2–6.8 (8 H, $A_2B_2$ systems, aromatic), 4.55 (1 H, t, $J$ = 7 Hz, α-H), 3.8 (3 H, s, $OCH_3$), 2.0 (2 H, m, α-$H_2$), 0.89 (3 H, t, $J$ = 7 Hz, α-$H_3$); *m/e* 299 ($M^+$, 100), 271 ($M^+$ − 18, 28).

**4-Amino-4′-methoxy-α-ethyldeoxybenzoin**[9] (3). 2 was reduced as described previously.[9] The recrystallized product (70% yield) was characterized as follows: mp 99–100° (lit.[9] 100–101°); $R_f$ 0.57 ($CH_2Cl_2$–acetone, 80:1); (VS) yellow-green; ir (KBr) 3470, 3380, 1600 ($NH_2$), 1670 (C=O); *m/e* 269 ($M^+$).

**3-Anisyl-4-(*p*-aminophenyl)hexanol-3**[10] (4). The mixture of diastereomeric carbinols was synthesized by the procedure of Rubin and Wishinsky[10] in 88% yield: mp 111–114° (lit.[10] mp 113–114°); $R_f$ 0.41 ($CH_2Cl_2$–acetone, 80:1); (VS) deep violet; ir (KBr) 3520 (OH), 3440, 3360, 1617 ($NH_2$); *m/e* 299 ($M^+$, 42), 281 ($M^+$ − 18, 100).

**3-Anisyl-4-(*p*-nitrophenyl)hexanol-3** (5). By analogy to the oxidation procedure of Yost[11] a solution of 6.1 g (0.035 mol) of *m*-chloroperoxybenzoic acid in 300 ml of $CHCl_3$ was added to 1.8 g (0.006 mol) of 4 in 60 ml of $CHCl_3$ at 0° over a period of 15 min and the solution was allowed to stand for 30 min at room temperature. Tlc showed that the major part of the amine was oxidized. The reaction mixture was extracted with 15-ml portions of 0.5 *N* NaOH until it became neutral and was then washed with water. The oily residue (approximately theoretical yield) which remained after evaporation of the solvent appeared pure on tlc and was used without further purification: $R_f$ 0.78 ($CH_2Cl_2$–$Et_2O$, 80:7); (VS) violet; ir ($CHCl_3$) 3500 (OH), 1510, 1345 ($NO_2$); nmr ($CDCl_3$) 7.95–6.55 (8 H, $2A_2B_2$ systems, aromatic), 3.55 (3 H, s, $OCH_3$), 2.8 (1 H, dd, $J_{4,5}$ = 3.5 and 10.5 Hz, 4-H), 2.35 (1 H, s, OH), 2.1–1.5 (4 H, m, 2-$H_2$, 5-$H_2$), 0.65 (3 H, t, $J_{1,2}$ = 7 Hz, 1-$H_3$), 0.50 (3 H, t, $J_{5,6}$ = 7 Hz, 6-$H_3$); *m/e* 329 ($M^+$, 4), 311 ($M^+$ − 18, 41), 300 ($M^+$ − 29, 100).

**4-Nitro-4′-methoxy-7,7′-diethyl-*trans*-stilbene** (6). 5 (7.35 g, 0.022 mol) was refluxed 6 hr in 250 ml of a mixture of glacial acetic acid–concentrated HCl–$H_2O$ (10:5:1 by volume).[12] The cooled mixture was extracted in portions with a total of 800 ml of $Et_2O$. The ethereal phases were extracted with 1 *N* NaOH until they were neutral, washed ($H_2O$), dried ($MgSO_4$), and evaporated under reduced pressure. To remove polar materials, the residue was chromatographed on a column (2.5 cm × 10 cm, 70 g of neutral aluminum oxide, 100–200 mesh, Biorad), which was sequentially eluted with 800 ml of $CH_2Cl_2$–*n*-hexane (1:10) and 300 ml of $CH_2Cl_2$–*n*-hexane (1:1). After removal of the solvent under reduced pressure, the yield of the mixture of cis and trans isomers was 5.3 g (69%).

To isolate the pure trans isomer of 6, 3.2 g of the mixture was applied to a column (150 g of aluminum oxide, 2.5 × 20 cm) and sequentially eluted with about 1000 ml of 2% $CH_2Cl_2$, 500 ml of 5% $CH_2Cl_2$, and 300 ml of 10% $CH_2Cl_2$ in *n*-hexane. The oily residue which remained after removal of the solvent from the first band crystallized overnight at −10° (6, 1.3 g, 28%). Recrystallization (*n*-hexane) furnished yellow-green needles (1 g, mp 83°). *Anal.* ($C_{19}H_{21}NO_3$) C, H, N, O. The cis product emerged as the second band from the column and was an oil which did not crystallize at −10°. *trans*-6: ir (KBr) 1510, 1345 ($NO_2$); nmr 8.40–6.85 (8 H, $A_2B_2$ systems, aromatic), 3.85 (3 H, s, $OCH_3$), 2.25 (2 H, q, $J_{8,9}$ = 7 Hz, 8-$H_2$), 2.15 (2 H, q, $J_{8,9}$ = 7 Hz, 8′-$H_2$), 0.78 (6 H, t, $J_{8,9 \text{ or } 8',9'}$ = 7 Hz, 9′ and 9-$H_3$); *m/e* 311 ($M^+$, 60), 296 ($M^+$ − 15, 48), 280 ($M^+$ − 31, 100), 236 ($M^+$ − 75, 32), 221 ($M^+$ − 90, 37).

**4-Nitro-4′-hydroxy-7,7′-diethylstilbene** (7). 6 (4.8 g, 0.016 mol) (cis and trans mixture) was refluxed in 46 ml of acetic acid–48% HBr (1:1 v/v) for 2 hr.[9] Water (1 vol) was added to the cooled reaction mixture which was then extracted with $Et_2O$ (600 ml). The ethereal phases were neutralized with 1 *N* NaOH as described for 6. The residue was then applied to a 50-g column of aluminum oxide. *n*-Hexane–$CH_2Cl_2$ (200 ml, 1:1) eluted unreacted 6, while 7 was eluted with 150 ml of MeOH. The residual brownish resin (3.1 g, 87%) appeared homogeneous on tlc: $R_f$ 0.69 ($CH_2Cl_2$–acetone, 80:5); (VS) violet. The mixture of cis and trans isomers of 7 did not crystallize and was used without further purification: ir ($CHCl_3$) 3600 (OH), 1510, 1345 ($NO_2$); *m/e* 297 ($M^+$ 100), 282 ($M^+$ − 15, 20), 268 ($M^+$ − 29, 70).

**4′-Hydroxy-7,7′-diethyl-4-stilbenylhydroxylamine** (8). 7 (4.9 g, 0.017 mol) was dissolved in 98 ml of dimethylformamide and saturated with $NH_3$ and $H_2S$[13] at 0°. After 4 hr at 0° crystalline $NH_4SH$ was removed by filtration and the reaction mixture was then added to 5 vol of $N_2$-flushed ice-cold water. The oily precipitate 8 was collected by centrifugation and dried over $P_2O_5$ under vacuum. Tlc showed one major spot which tailed toward the origin: $R_f$ *ca.* 0.2 ($CH_2Cl_2$–acetone, 80:7); (VS) violet-red. The viscous oil was used without further purification.

***N*-Acetoxy-4′-acetoxy-7,7′-diethyl-*trans*-*N*-4-stilbenylacetamide** (9). 8, prepared from 4.9 g (0.017 mol) of 7, was dissolved in 50 ml of absolute pyridine. $Ac_2O$ (10 ml, 0.106 mol) was added dropwise at 0° under $N_2$. After 2 hr at room temperature 3 vol of water was added, and the reaction mixture was extracted in portions with a total of 500 ml of $CH_2Cl_2$. Pyridine was removed by 50-ml extractions with 1 *N* HCl, 5% aqueous $KHCO_3$, and $H_2O$, and the organic phase was dried ($MgSO_4$). After removal of the solvent the residue (5.5 g) was used for preparation of 10 without further purification. To obtain an analytical sample of 9 50 mg of the resin was applied on a silica gel thick-layer plate and developed four times with $CH_2Cl_2$–acetone (80:1). The compound in the least polar band (VS, dark violet) was identified as the triacetate 9 by its mass and ir spectra (see below). Pure crystalline *N*-acetoxy-4′-acetoxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide was obtained by acetylation of either *N*-hydroxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide (10) or *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide (11) with $Ac_2O$–pyridine (see below). $R_f$ values and ir and mass spectra of the triacetate obtained *via* the three different methods were identical. 9 (trans): $R_f$ 0.69 ($CH_2Cl_2$–acetone, 4:1); (VS) dark violet; mp 119.5° ($Et_2O$–*n*-hexane); ir (KBr) 1800 (NOC=O), 1770 (COC=O), 1690 (NC=O); nmr ($CDCl_3$) 7.6–7.0 (8 H, $A_2B_2$ systems, aromatic), 2.3 (3 H, s, $NCOCH_3$), 2.2 (3 H, s, $COCOCH_3$), 2.1 (3 H, s, $NOCOCH_3$), 2.3–2.0 (4 H, m overlapped, 8,8′-$H_2$), 0.75 (6 H, t, $J_{8,9 \text{ or } 8',9'}$ = 7 Hz, 9 and 9′-$H_3$); *m/e* 409 ($M^+$, 27), 367 ($M^+$ − 42, 23), 351 ($M^+$ − 58, 16), 325 ($M^+$ − 84, 18), 309 (26), 265 (100). *Anal.* ($C_{24}H_{27}NO_5$) H, N, O; C: calcd, 70.40; found, 69.92.

***N*-Hydroxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide** (10). Crude *N*-acetoxy-4′-acetoxy-7,7′-diethyl-*N*-4-silbenylacetamide (12 g, prepared from 10.6 g of 7) was dissolved in 150 ml of EtOAc and vigorously stirred with an equal volume of concentrated $NH_4OH$–$H_2O$ (v/v, 1:1) for 90 min at room temperature. The EtOAc was evaporated, and the residue was dissolved in 200 ml of 1 *N* NaOH and extracted with 50-ml portions of $Et_2O$ until the $Et_2O$ phases were colorless. The NaOH solution was cooled in ice and acidified to pH 3 with concentrated HCl. The oily precipitate was extracted with 600 ml of EtOAc, which

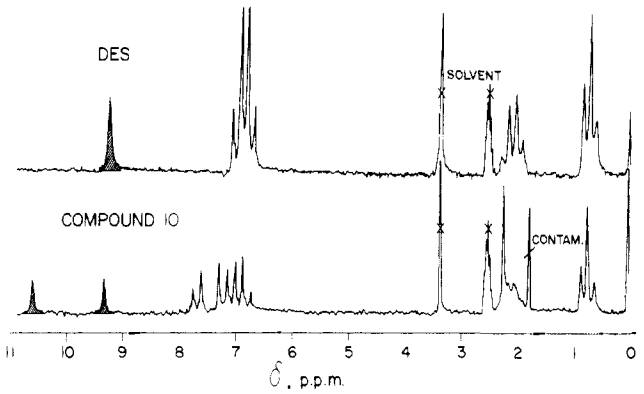


**Figure 1.** Nmr spectra (DMSO-$d_6$) of diethylstilbestrol (DES) and *N*-hydroxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-silbenylacetamide (10).

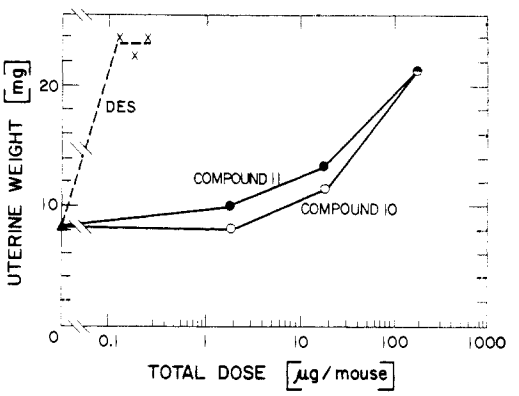


**Figure 2.** Uterine weights of immature mice after ip injections of diethylstilbestrol (DES), 10, and 11.

was neutralized with 5% aqueous $KHCO_3$ solution, washed, dried, and evaporated. The residual white powder (5.8 g) was applied to a column [325 g of silica gel PF254 (Merck), 3 × 58 cm] which was developed at 4° in the dark with $CH_2Cl_2$–acetone (80:7). 10 (trans) was eluted as the second band; it was preceded by an orange-yellow zone which was discarded. 10 (1.2 g) was isolated. The overall yield from 201 g of 1 was 2.7 g (1.1%). Recrystallization (EtOH–water, 2:1, 3 times, or acetone–$Et_2O$–*n*-hexane) gave white crystals, mp 130–133°. Possibly because of solvent inclusion no correct elementary analysis for C and H [*Anal.* ($C_{20}H_{23}NO_3$) N, O; C: calcd, 73.82; found 72.88; H: calcd, 7.12; found, 7.87] was obtained for 10 even after it was acetylated with $Ac_2O$ in pyridine to the triacetyl compound 9 and then hydrolyzed again to 10 (mp 135–137°). On high-resolution mass spectrometry the molecular ion of 10 was observed at *m/e* 325.1682 (calcd 325.1678): $R_f$ 0.46 ($CH_2Cl_2$–acetone, 4:1); (VS) violet-red; ir (KBr) 3220 i, 3160 (OH), 1660 i, 1640 (C=O); nmr (Figure 1) 10.53 (1 H, s, NOH), 9.27 (1 H, s, OH), 7.75–6.6 (8 H, $A_2B_2$ systems, aromatic), 2.20 (3 H, s, $COCH_3$), 2.3–1.95 (4 H, m overlapped, 8 and 8′-$H_2$), 0.70 (6 H, t, $J_{8,9\text{ or }8',9'}$ = 7 Hz, 9 and 9′-$H_3$); *m/e* 325 ($M^+$, 100), 309 ($M^+ - 16$, 11), 283 ($M^+ - 42$, 62), 266 ($M^+ - 59$, 61).

***N*-Acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide (11).** To 100 mg (0.31 mmol) of 10 dissolved in 6 ml of absolute pyridine, 0.31 mmol of $Ac_2O$ in 6 ml of pyridine was added dropwise under $N_2$. After 1 hr at room temperature 2 vol of water was added, and the reaction mixture was extracted in portions with a total of 225 ml of EtOAc. After work-up as described for 9 the resin crystallized spontaneously (91 mg, 80%). After three recrystallizations (EtOH–water) white crystals (37 mg, 32%) were obtained. The melting point was 146–149° dec when measured on a Fisher-Johns melting point apparatus. On a rapid time–temperature curve 11 showed only an exothermic inflection at 172° due to ortho rearrangement of the acetoxy group:[14] $R_f$ 0.51 ($CH_2Cl_2$–acetone, 80:5); (VS) violet-red; ir (KBr) 3220 (OH), 1805 (NOC=O), 1650 (NC=O); uv (EtOH) 231, 252 i (log ε 4.76); nmr (DMSO-$d_6$) 9.32 (1 H, s, OH, exchangeable), 7.6–6.6 (8 H, $A_2B_2$ systems, aromatic), 2.25 (3 H, s, $NCOCH_3$), 2.03 (3 H, s, $NOCOCH_3$), 2.4–1.9 (4 H, m overlapped, 8 and 8′-$H_2$), 0.70 (6 H, t, $J_{8',9'}$ or $J_{8,9}$ = 7 Hz, 9 and 9′-$H_3$); *m/e* 367 ($M^+$, 77), 351 ($M^+ - 16$, 12), 325 ($M^+ - 42$, 41), 309 ($M^+ - 58$, 47), 283 ($M^+ - 84$, 22), 265 (100). *Anal.* ($C_{22}H_{25}NO_4$) C, H, N, O.

**Acetylation of 10 and 11 to Give 9.** 10 (150 mg) (or 55 mg of 11) was dissolved in 10 ml (6 ml) of absolute pyridine, and $Ac_2O$ (5 molar excess) in 1 ml of pyridine was added under $N_2$ at 0°. After 2 hr at room temperature, 2 vol of water were added, and the reaction product was worked up as described for 10. The resins from both reactions were applied to thick layer chromatograms ($CH_2Cl_2$–acetone, 80:7). The main product 9 was extracted from the gel with EtOAc and crystallized spontaneously upon evaporation of the solvent: yield, 144 mg, 77% (46 mg, 75%). Analytical data are listed under 9.

**Assay for Estrogenic Activity.**[15] Groups of ten immature CF-1 (Carworth Farms) female mice weighing 12–16 g were injected ip once each day for 3 days with 0.05 ml of DMSO which contained diethylstilbestrol (Matheson Coleman and Bell) (0.04, 0.06, or 0.08 μg) or 10 or 11 (0.6, 6, or 60 μg). On the fourth day the mice were killed and the uteri were excised, freed of intrauterine fluid, and weighed in groups of three or four.[15] Uteri from control mice injected only with dimethyl sulfoxide or with no treatment had average weights of 7.6 and 8.6 mg, respectively.

**Assay for Substitution of *N*-Acetoxy-*N*-arylacetamides by Methionine-*methyl*-$^3H$.** The following ingredients were incubated in a 1-ml volume for 2 hr at 37° under $N_2$: 0.5 ml of M/15 Sorensen phosphate buffer, pH 7.0; 0.25 μmol of hydroxamic acid ester dissolved in 0.4 ml of acetone; 0.38 nmol of L-methionine-*methyl*-$^3H$ (3.3 Ci/mmol, Schwarz-Mann, Inc.).[16] Each assay mixture was then extracted with 7 ml of 30% PhH in *n*-hexane to remove the residual *N*-acetoxy derivative, heated at 90° for 45 min to ensure the decomposition of the sulfonium derivative, cooled, brought to pH 9–10 with 11 *N* KOH, and extracted with 7 ml of 30% PhH in *n*-hexane. After being washed and dried ($MgSO_4$) the combined organic phases were assayed for $^3H$ in a Packard Tri-Carb scintillation counter. The blank value of 0.56%, obtained in the absence of any electrophilic derivative, was subtracted from the values reported.

## Results and Discussion

**Chemistry.** The synthetic route to the desired compounds included the synthesis of 4-nitro-4′-methoxy-7,7′-diethylstilbene (a new compound). This compound was then demethylated, reduced, and acetylated in a stepwise fashion to yield *N*-hydroxy- and *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-stilbenylacetamide.

Resolution of the cis–trans mixtures was effected at two stages of the syntheses, and the configurations of the trans isomers of 7, 9, 10, and 11 were established by nmr measurements. As reported previously for other 7,7′-diethylstilbene derivatives, the multiplets arising from the ethyl groups of the cis and trans isomers are separated ($\Delta\delta$ ~0.2), and the resonances of the trans products occur at higher field than those of the cis.[17,18] The chemical shifts for the multiplets for the two ethyl groups of trans 7, 9, 10, and 11 were the same as those reported for diethylstilbestrol (see Figure 1).

In spite of our inability to obtain a sample of *N*-hydroxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide (10) with the correct elementary analysis this compound was characterized unequivocally by spectroscopic means. High-resolution mass spectrometry showed the appropriate mass for the molecular ion. Fragments were observed at $M^+ - 16$ and $M^+ - 42$; these fragments have been frequently observed in the mass spectra of aromatic acethydroxamic acids.[19] The absorption peaks for two hydroxyl groups were located in the ir spectrum at 3270 and 3160 $cm^{-1}$. The nmr spectrum (Figure 1) showed the signals for two $D_2O$-exchangeable hydroxyl groups; the trans configuration of 10 was evident from the chemical shift (0.70 ppm) of the methyl groups (triplets).

**Estrogenic Activity.** *N*-Hydroxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide (10) and its *N*-acetoxy derivative 11 each exhibited significant estrogenic activity (Figure 2). These activities were 1/600 and 1/400, respectively, that of diethylstilbestrol, but these lower activities were expected in view of the well-known importance of

the two hydroxyl groups for the estrogenic potency of diethylstilbestrol.[20,21] Replacement of one of the hydroxyl groups in diethylstilbestrol by an amino group had been shown to reduce the estrogenic activity 25-fold, and larger substitutions (methoxy or bromine) caused much greater reductions in activity.[10]

**Electrophilic Reactivity.** The reaction of esters of *N*-arylacethydroxamic acids with methionine-*methyl*-$^3H$ to yield sulfonium derivatives which decompose to benzene-soluble [$^3$H]methylmercaptoarylamides has served as a convenient assay for the electrophilic reactivity of these compounds.[14] Under our assay conditions 0.65% of the $^3H$ in methionine-*methyl*-$^3H$ was converted to a benzene-hexane-soluble form on incubation with *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide. Under the same conditions reactions of 0.58 and 5.3% were obtained with *N*-acetoxy-*N*-4-*trans*-stilbenylacetamide or *N*-acetoxy-*N*-2-fluorenylacetamide, respectively. From the similar activity of **11** and *N*-acetoxy-*N*-4-stilbenylacetamide toward methionine, **11** might be expected to be reactive toward certain nucleophilic sites in cellular macromolecules. Carcinogenicity tests on *N*-hydroxy- and *N*-acetoxy-4′-hydroxy-7,7′-diethyl-*N*-4-*trans*-stilbenylacetamide are in progress.

**Acknowledgment.** The work was supported by Grants CA-07175 and CRTY-5002 of the National Cancer Institute, U. S. Public Health Service. H. B. was a recipient of a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft, Germany. We are indebted to Mr. R. Weisenberger, Department of Chemistry, Ohio State University, Columbus, Ohio, for the high-resolution mass spectrum.

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# Total Synthesis of Bisnorpenicillin V

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Bisnorpenicillin V, an analog of penicillin V in which the two methyl groups are replaced by hydrogen atoms, was prepared by total synthesis. The reaction scheme was essentially that described by Sheehan, *et al.*, for the synthesis of penicillins. The antibacterial activity of bisnorpenicillin was lower than that observed for the parent penicillin V. Replacement of the two C-2 methyl groups of penicillin has no or little effect on its sensitivity to β-lactamase.

Several modifications at the C-2 position of penicillins have been reported recently.[1-4] These modifications concern the introduction of an acetoxy group or a halogen into one of the C-2 methyl groups. The present report deals with the synthesis of bisnorpenicillin V (**1a**), a penicillin V analog in which the two C-2 methyl groups are replaced by hydrogen atoms.† From earlier work[6] in the field of total synthesis of penicillins, one would expect bisnorpenicillins to be biologically active. Antibacterial activity was found for the reaction mixture of DL-cysteine with 2-benzyl-4-methoxymethylene-5(4)-oxazolone, a condensation which yields penicillin in very low yields.

For the preparation of pure **1a** the two reaction schemes reported by Sheehan, *et al.*,[7,8] for the synthesis of penicillin V (**1b**) were followed. Both reaction sequences, outlined in reaction schemes A and B, start with *tert*-butyl (3-carboxy-5-thiazolidine)phthalimidoacetate (**2a**).‡ In order to obtain a biologically active penicillin, it is necessary to use the isomer of **2a** with the configuration of natural penicilloate. This isomer can be obtained by condensation of *tert*-butyl phthalimidomalonaldehyde[9] (**3**) with D-cysteine in aqueous ethanol containing sodium acetate. The reaction proceeds in a similar way as reported for the condensation of **3** with D-penicillamine[7] and afforded a mixture of two diastereoisomers of **2a** in a ratio of about 5:1. In analogy to the synthesis of **2b**,[7] the natural configuration was assigned to the minor component, which will be designated as α isomer (the major component as γ isomer). The α and γ isomers can be differentiated by tlc (silica gel, solvent system I). In both cases the α isomer showed

† According to the nomenclature, proposed by Sheehan, *et al.*,[5] compound **1a** should be called 6-phenoxyacetamidopenam-3-carboxylic acid.

‡ To avoid confusion the penam numbering will be used in this paper for the thiazolidine nucleus of compounds related to penicillin.