JOC Notes

Convenient Synthesis of 5,6,11,12,17,18-Hexahydrocyclononal[1,2-*b*:4,5-*b*′:7,8-*b*″]triindole, a Novel Phytoestrogen

Richard E. Staub and Leonard F. Bjeldanes*

Department of Nutritional Sciences and Toxicology, University of California, Berkeley, California 94720-3104

lfb@nature.berkeley.edu

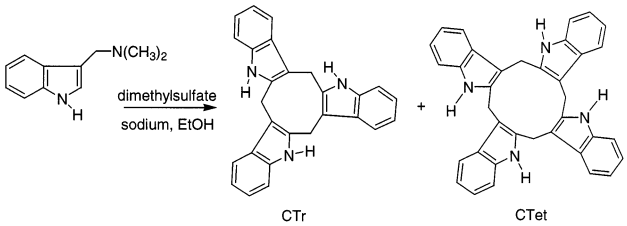
Received June 18, 2002

Abstract: An efficient one-pot synthesis is described of 5,6,11,12,17,18-hexahydrocyclononal[1,2-*b*:4,5-*b*′:7,8-*b*″]triindole (CTr), a potent estrogen agonist from food plants. For the procedure, gramine is treated with dimethyl sulfate and sodium in ethanol at room temperature. Quenching of the reaction with water and workup of the product provides CTr in approximately 75% yield.

5,6,11,12,17,18-Hexahydrocyclononal[1,2-*b*:4,5-*b*′:7,8-*b*″]triindole (CTr) is a novel estrogen agonist produced under acidic conditions from the putative cancer protective agent indole-3-carbinol (I3C). I3C is an enzymatic hydrolysis product of the indolylmethyl glucosinolate glucobrassicin that occurs in common food plants of the genus *Brassica*, including cabbage, kale, cauliflower, Brussels sprouts, and broccoli. Under acidic conditions in vitro, and following ingestion, I3C readily self-condenses to form a mixture of oligomeric products including CTr.[1−3] I3C and its oligomeric products are under study as cytostatic[4−7] and tumor-suppressive agents.[8] In addition, I3C exhibits tumor-promoting activity in some assays,[9] an effect that may arise in part from estrogenic products of I3C formed in vivo. We have shown that CTr is a strong agonist of estrogen receptor function. Computational modeling studies indicated an excellent fit of CTr into the ligand-binding site of the estrogen receptor.[7]

For our continued studies of the biological effects of CTr, we required a high-yielding synthetic procedure for CTr and possible analogues. Currently the synthetic procedure for CTr is a low-yielding process that requires HPLC purification of product from the complex acid reaction mixture of I3C.[7] We report a convenient, high-yield, one-pot procedure for the synthesis of CTr in quantities required for metabolic and structure−activity studies.

SCHEME 1



Treatment of I3C under a variety of conditions provided either a complex mixture of oligomeric products (acidic conditions) or a reasonably good yield of the dimeric product 3,3′-diindolylmethane (basic conditions). We then turned our attention to gramine as a source of the presumed 3-methylene indolenine oligomerization intermediate. Although the literature is extensive on the use of gramine quaternary ammonium salt in the synthesis of 3-methylindole analogues,[10] we are aware of no reports of the preparation of self-condensation products from this starting material. We used dimethyl sulfate to generate the quaternary salt of gramine in the presence of sodium ethoxide (Scheme 1), conditions that presumably produced the reactive indolenine intermediate. The reaction was surprisingly facile and yielded primarily CTr (∼75%) with only one significant byproduct, the cyclic tetramer (CTet, ∼9%) (Figure 1).

Spectral analysis provided full support for the proposed structure of CTr and its conformational preferences. The ^{1}H NMR spectrum for CTr (Figure 2) was similar to that previously reported[2] and demonstrates that this compound is composed of almost equal proportions of crown and saddle conformations, on the basis of the resonances of the saturated cyclononane methylene protons.[11,12] This spectral information, which is corroborated with Dreiding models, indicates that the geminal methylene protons are fixed in position in the crown conformation, resulting in two doublets in the ^{1}H NMR spectrum.[11] In contrast, the cyclononane ring in the saddle conformation has considerable flexibility, which collapses the ^{1}H NMR signals for the six methylene protons to a singlet.[2,12] ^{13}C NMR analysis of CTr prepared with ^{13}C-2-labeled precursor also gave two enriched signals, presumably for the crown and saddle conformations. Further, the natural-abundance ^{13}C NMR spectrum for CTr shows splitting for the resonances of C-2, C-3, C-9, and the methylene carbon

(1) De Kruif, C. A.; Marsman, J. W.; Venekamp, J. C.; Falke, H. E.; Norrdhoek, J.; Blaauboer, B. J.; Wortelboer, H. M. *Chem.-Biol. Interact.* **1991**, *80*, 303−315.
(2) Grose, K. R.; Bjeldanes, L. F. *Chem. Res. Toxicol.* **1992**, *5*, 188−193.
(3) Stresser, D. M.; Williams, D. E.; Griffin, D. A.; Bailey, G. S. *Drug Metab. Dispos.* **1995**, *23*, 965−975.
(4) Ge, X.; Yannai, S.; Rennert, G.; Gruener, N.; Fares, F. A. *Biochem. Biophys. Res. Commun.* **1996**, *228*, 153−158.
(5) Chang, Y.-C.; Riby, J.; Chang, G. H−F.; Peng, B.-C.; Firestone, G.; Bjeldanes, L. F. *Biochem. Pharm.* **1999**, *58*, 825−834.
(6) Riby, J. E.; Chang, G. H. F.; Firestone, G. L.; Bjeldanes, L. B. *Biochem. Pharm.* **2000**, *60*, 167−177.
(7) Riby, J. E.; Feng, C.; Chang, Y.-C.; Schaldach, C. M.; Firestone, G. L.; Bjeldanes, L. F. *Biochemistry* **2000**, *39*, 910−918.
(8) Chen, I.; McDougal, A.; Wang, F.; Safe, S. *Carcinogenesis* **1998**, *19*, 1631−1639.
(9) Stoner, G.; Casto, B.; Ralston, S.; Roebuck, B.; Pereira, C.; Bailey, G. *Carcinogenesis* **2002**, *23*, 265−272.
(10) Sundberg, R. J. *Indoles;* Academic Press Inc.: San Diego, CA, 1996.
(11) Erdtman, H.; Haglid, F.; Ryhage, R. *Acta Chem. Scand.* **1964**, *18*, 1249−1254
(12) Bergman, J.; Högberg, S.; Lindström, J.-O. *Tetrahedron* **1970**, *26*, 3347−3352.

10.1021/jo020415y CCC: $25.00
Published on Web 12/06/2002

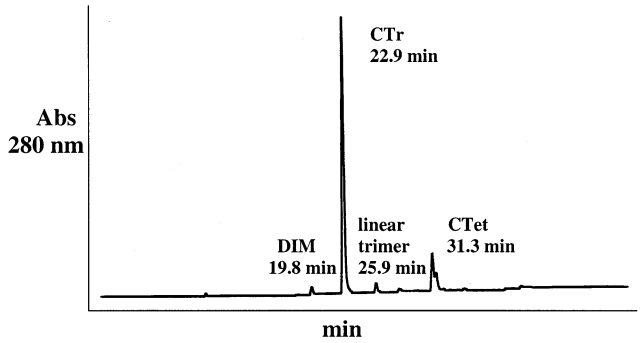


FIGURE 1. Reversed-phase HPLC chromatogram (280 nm) displaying the distribution of products from the synthesis of cyclic trimer from gramine.

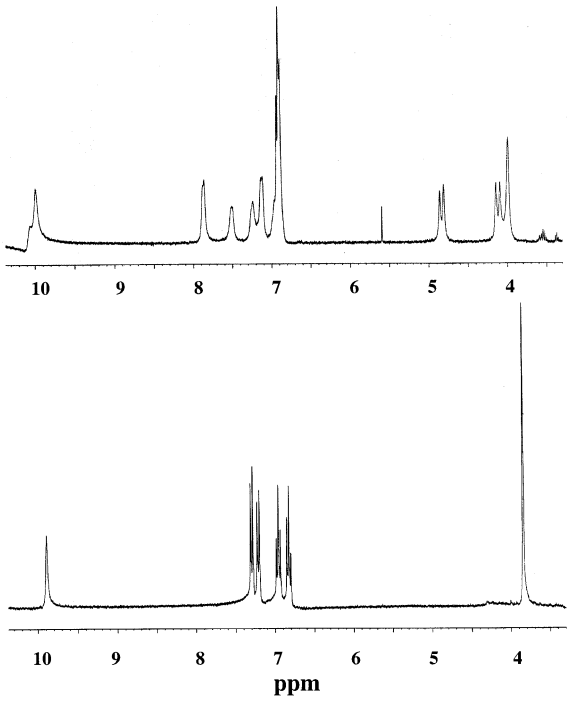


FIGURE 2. ^{1}H NMR spectra in deuterated acetone for CTr (top) and CTet (bottom).

as would be expected for the saddle and crown conformations. The resonance for C-8 (based on the ^{13}C NMR spectrum for indole[13]) was not detected and is probably under the C-2 resonance since the same resonances were separated by less than 0.5 ppm in the CTet spectra. Only single resonances were observed for the phenyl carbons, whereas two signals were recorded for the other carbons representing the crown and saddle conformations.

^{1}H NMR analysis of the [^{13}C]CTr analogue resulted only in a broadening of the methylene proton signals, consistent with the C-2,C-3′-methylene bridge and the *all-anti*-indole ring structural assignments. Mass spectral analysis provided the expected molecular mass of 387 (observed as 388 for the M + 1 ion), which increased by 3 mass units when ^{13}C-labeled precursor was included in the synthesis reaction, thereby verifying the presence of three indole rings in the product.

The ^{1}H NMR spectrum for the CTet (Figure 2) was similar to that previously reported[12] with all eight methylene protons represented as a singlet at 3.85 ppm (acetone-d_6) consistent with its flexible structure. ^{13}C NMR analysis of CTet prepared from ^{13}C-2-labeled precursor gave a single enriched signal, also consistent with its flexible structure. Further, ^{1}H NMR analysis of the ^{13}C-labeled CTet resulted in a broad multiplet at 3.85 ppm, thereby verifying the C-2,C-3′-methylene bridges between the indoles in the ring. Mass spectral analysis of the CTet product provided the expected molecular mass of 516 (observed as 517 for the M + 1 ion), which increased by 4 mass units when ^{13}C-labeled precursor was used, thereby indicating the presence of four indole rings in the product.

The same synthetic procedure could be applied utilizing other alcoholic solvents (methanol and 2-propanol), but the reaction was not as efficient for the CTr product (50−52%). Likewise, the synthesis of cyclic trimer from indole-3-carbinol (with dimethyl sulfate and sodium) was less efficient (44.7 ± 2.8%, n = 3) and resulted in a greater amount of the CTet (11.1 ± 1.7%, n = 3), linear trimer (7.2 ± 1.2%, n = 3), 3,3′-diindolylmethane (3.5 ± 0.5%, n = 3), and other byproducts as determined by HPLC analysis. The CTr product was similarly produced when sodium ethoxide was first prepared in ethanol and then added to the quarternary ammonium intermediate. However, this reaction was again not as efficient as when the sodium ethoxide was generated in the presence of the gramine intermediate. No 3-ethoxymethylindole was produced under our standard reaction conditions involving the formation of the quarternary ammonium intermediate, even though this was reported to be the primary product when gramine was reacted with an alkylating agent (methyl or ethyl iodide) and sodium ethoxide in ethanol.[14]

Facile cyclononane ring formation from gramine apparently has some precedent in the preparation of cyclotriveratrylene from veratrole or veratrylamine *N*-tosylates.[11,15,16] Further, a minor byproduct of the cyclotriveratrylene condensation is the corresponding cyclic tetramer. In contrast to the cyclotriveratrylene condensation that is formed under highly acidic conditions, our gramine-based procedure is catalyzed by strong base. Further studies of this novel reaction should clarify its unexpected product specificity and may prove useful in the preparation of a unique group of estrogen receptor agonists and antagonists of potential therapeutic usefulness.

Experimental Section

General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz, and chemical shifts are reported in parts per million from that of the deuterated solvent. Commercial gramine and I3C were recrystallized from toluene prior to use in the synthesis procedures.

Preparative reversed-phase chromatography was performed using reversed-phase HPLC (C-18, 10 × 250 mm, 5 μm). A gradient elution was used: initially at 60% acetonitrile followed

(13) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH Publishers: New York, 1987.

(14) Geissman, T. A.; Armen, A. *J. Am. Chem. Soc.* **1952**, *74*, 3916−3919.

(15) Lindsey, A. S. *J. Chem. Soc.* **1965**, 1685−1692.

(16) Umezawa, B.; Hoshino, O.; Hara, H.; Ohyama, K.; Mitsubayashi, S.; Sakakibara, J. *Chem. Pharm. Bull.* **1969**, *17*, 2240−2244.

by linear gradients of 60−95% acetonitrile over 40 min, 95−100% over 5 min, and then constant 100% for 5 min before returning to initial conditions. The eluent was monitored at 280 nm for collection of appropriate products. Retention for products was as follows: CTr, 22.9 min; CTet, 31.3 min; 3,3′-diindolylmethane, 19.8 min; linear trimer, 25.9 min.

For LC/MS analysis, isocratic acetonitrile (75%) and aqueous 0.1% formic acid were pumped at a 0.2 mL/min flow rate (4.6 × 250 mm, 5 μm), and the eluent was monitored at 280 nm. The electrospray source was operated at 5.5 kV potential and a heated capillary temperature of 250 °C. Nitrogen gas was used at a sheath gas pressure of 40 psi. Auxiliary gas flow was adjusted to achieve a total flow of 0.5 L/min of nitrogen. For MS/MS experiments, argon (1 Torr) was used as the collision gas with a collision energy of −25 V.

Synthesis of CTr. Dimethyl sulfate (1.2 mL, 12.6 mmol) was added to gramine (0.5 g, 2.88 mmol) dissolved in 30 mL of absolute ethanol. Solid sodium (100 mg, 4.3 mmol) was added, and the solution was constantly stirred at room temperature for 4 h. The reaction mixture was poured into 100 mL of water and partitioned with dichloromethane (50 mL, 2×). The combined organic layers were back-extracted with 5% sodium carbonate (75 mL) and water (100 mL), dried over Na_2SO_4, filtered, and evaporated in vacuo. Before dryness was reached, 25 mL of hexane was added to precipitate the product and produce a yellow-white final powder when dry. The mass yield was nearly quantitative (97.1 ± 2.7%, $n = 3$) for a crude product that contained ∼75% CTr and ∼9% CTet as determined by HPLC analysis. Trace amounts of linear trimer and 3,3′-diindolylmethane were also detected and verified by LC/MS as previously described.[2] Pure CTr for spectral analysis was only obtained by preparative reversed-phase HPLC. The CTr and CTet could not be separated using normal-phase chromatography (HPLC and TLC). Attempts to crystalize CTr were unsuccessful and were likely inhibited by the mixture of the saddle and crown conformations. CTet was crystallized from dimethyl sulfoxide as fine white crystals.

Data for CTr. ^{1}H NMR ($(CD_3)_2CO$): δ 3.99 (br s, CH_2 saddle), 4.12 (br d, $J = 15.5$ Hz, CH_2 crown), 4.83 (br d, $J = 15.0$ Hz, CH_2 crown), 6.91 (br m, ArH), 7.12 (br m, ArH), 7.24 (br m, ArH), 7.52 (br m, ArH), 7.87 (br m, ArH), 10.04 (br, NH from crown and saddle conformers). ^{13}C NMR ($(CD_3)_2CO$): δ 136.8 and 136.1 (br, C-2, crown and saddle), 129.8 and 129.5 (br, C-9, crown and saddle), 121.3 (C-4), 119.5 (C-5), 118.5 (C-6), 111.4 (C-7), 109.2 and 108.6 (br, C-3, crown and saddle), 22.65 and 21.72 (br, CH_2, crown and saddle). LC/MS (*m*/*z*): 388 (M + 1, 100). LC/MS/MS (*m*/*z*): products of 388; 388 (M + 1, 51), 271 (100), 257 (32), 245 (4), 130 ($C_8H_6N{=}CH_2^+$, 37). Mp: chars at 290 °C.

Data for CTet. ^{1}H NMR ($(CD_3)_2CO$): δ 3.85 (s, CH_2), 6.83 (t, $J = 7.0$ Hz, ArH), 6.96 (t, $J = 7.0$ Hz, ArH), 7.22 (d, $J = 7.7$ Hz, ArH), 7.30 (d, $J = 8.0$ Hz, ArH), 9.91 (s, NH). ^{13}C NMR (pyridine-d_5): δ 137.3 (C-8), 136.9 (C-2), 130.6 (C-9), 121.3 (C-4), 119.6 (C-5), 118.7 (C-6), 111.9 (C-7), 108.3 (C-3), 22.81 (CH_2). LC/MS (*m*/*z*): 517 (M + 1, 100). Mp: chars at over 300 °C.

[^{13}C-2]Indole was used to synthesize [^{13}C-2]I3C as previously described.[17] [^{13}C-2]I3C was then reacted under the conditions described above to produce ^{13}C-labeled cyclic products for spectral analysis.

Data for [^{13}C-2]CTr. ^{13}C NMR ($(CD_3)_2CO$): δ 136.1 (s), 135.3 (s). LC/MS (*m*/*z*): 391 (M + 1, 100). LC/MS/MS (*m*/*z*): products of 391; 391 (M + 1, 9), 389 (12), 273 (100), 258 (36), 247 (17), 131 ($C_8H_6N{=}CH_2^+$, 70).

Data for [^{13}C-2]CTet. ^{13}C NMR ($(CD_3)_2CO$): δ 136.0 (s). LC/MS (*m*/*z*): 521 (M + 1, 100).

Acknowledgment. We thank Nanjing Zhang and Professor Casida at the University of California at Berkeley for their assistance in obtaining the ^{13}C NMR spectra. This research was supported in part by the Department of Defense, Army Breast Cancer Research Program Grant DAMD17-96-1-6149, National Institutes of Health Grant CA69056, and a National Institutes of Health, NRSA Trainee appointment on Grant 5-T32 ES07075.

JO020415Y

(17) Staub, R. E.; Feng, C.; Onisko, B.; Bailey, G.; Firestone, G. L.; Bjeldanes, L. F. *Chem. Res. Toxicol.* **2002**, *15*, 101−10.