removed (below 30°). Chromatography [Al_2O_3; eluent Et_2O (10%) in light petroleum] of the residue gave the furazan 1 (2.5 g, 55%) as pale buff needles: mp 140° (decomposing, depending on the rate of heating, above *ca.* 100°); ir (Nujol and $CHBr_3$) 1615 (w), 1600 (w), 1480 (m), 1410 (m), 1350 (m), 1315 (m), 1200 (m), 1150 (w), 1040 (w), 1020 (m), 940 (w), 820 (s), 810 (s), 770 (s); nmr τ_A 1.85, τ_B 2.00, τ_C 2.27 (J_{AC}, J_{BC} = 7 Hz).

Anal. Calcd. for $C_{12}H_6N_2O$: C, 74.2; H, 3.1; N, 14.4. Found: C, 74.5; H, 3.3; N, 14.1.

B.—Acenaphthofurazan oxide (2)[10] (0.1 g, 0.5 mmol) was allowed to stand for 48 hr with triethyl phosphite (5 g) at 20°. At the end of that period the solid had disappeared and the solution had become red-brown. It was poured into water (100 ml) containing 2–3 drops of HCl, and stirred until the smell indicated that the excess of phosphite had been hydrolyzed. Extraction (CH_2Cl_2) and chromatography on alumina as above gave the furazan 1 (0.01 g, 10%).

3-(8-Cyano-1-naphthyl)-5-phenylisoxazole (6).—The furazan 1 (0.1 g, 0.5 mmol) and phenylacetylene (0.07 g, 0.7 mmol) were heated to 125–130° for 15–20 min in xylene (3 ml). After cooling, the reaction mixture was chromatographed on alumina, eluting xylene and phenylacetylene with light petroleum, and then the adduct 6 with diethyl ether. The product formed needles (0.09 g, 55%): mp 142–143° (from ethanol); ir ($CHBr_3$) 3120 (m) (isoxazole CH), 2210 (m) (CN); nmr τ 3.22 (1 H, isoxazole), 1.8–2.6 (11 H).

Anal. Calcd for $C_{20}H_{12}N_2O$: C, 81.1; H, 4.05; N, 9.45. Found: C, 81.1; H, 4.25; N, 9.3.

1,8-Dicyanonaphthalene (3).—Heating the furazan 1 (0.1 g) to 80° for 4 hr with trimethyl phosphite (5 ml), followed by work-up in the usual way, gave the dinitrile 3 (95%), identical with a sample prepared previously[1] by reduction of the furazan oxide 2.

Registry No.—1, 206-28-0; 3, 5690-48-2; 4, 1932-08-7; 5, 37439-76-2; 6, 37439-77-3; phenylacetylene, 536-74-3.

The Structure and Partial Synthesis of Fabacein[1a,b]

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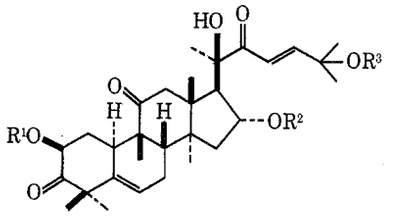
Fabacein is a bitter principle isolated from *Echinocystis fabacea* (Cucurbitaceae), and its isolation and preliminary characterization as a cucurbitacin diacetate derivative were described by Noller and coworkers.[2-4] In an extension of our recent studies of the structures of the cytotoxic cucurbitacins,[5-7] we have examined further the chemistry of fabacein. We report herein the structure elucidation and partial synthesis of fabacein (1), the first recognized naturally occurring cucurbitacin 16-acetate ester derivative.

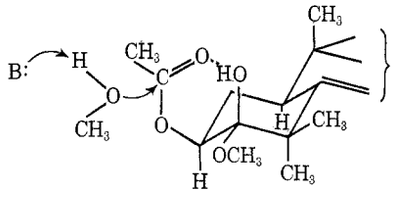


1, $R^1 = H$; $R^2 = R^3 = Ac$
2, $R^1 = R^2 = H$; $R^3 = Ac$
3, $R^1 = R^2 = R^3 = Ac$

Elemental analysis supported assignment of the molecular formula $C_{34}H_{48}O_9$[3] for fabacein (mp 198–201°, $[\alpha]^{25}D$ +36° absolute EtOH), and the spectral properties [$\lambda_{max}^{CHCl_3}$ 230 nm (ϵ 10,000); $\lambda_{max}^{CHCl_3}$ 2.91, 3.37, 3.43, 5.78, 5.91, 6.14, 6.84, 7.30, 8.00, 8.29, 8.87, 9.70, 10.2, and 10.8 μ; nmr ($CDCl_3$) τ 2.92 (1 H, d, J = 15 Hz), 3.68 (1 H, d, J = 15 Hz), 4.32 (1 H, m), 4.88 (1 H, b t, J = 8 Hz), 5.68 (1 H, d of d, J = 14, 6 Hz), 8.04 (3 H, s), 8.18 (3 H, s), 8.45 (3 H, s), 8.48 (3 H, s), 8.62 (3 H, s), 8.69 (3 H, s), 8.72 (3 H, s), 8.76 (3 H, s), 8.93 (3 H, s), and 8.99 (3 H, s); mass spectrum *m/e* 540, 445, 385, 369, 111, 96, and 43] supported its formulation as a cucurbitacin diacetate. The nmr signal at τ 4.88 (1 H, b t, J = 8 Hz) was characteristic of a C-16 proton in a 16-acetate derivative,[8] and, in view of the cooccurrence of fabacein with cucurbitacin B (2),[2] the hypothesis was entertained that fabacein is the 16-acetate ester (1) of cucurbitacin B (2).[3]

Interrelation of fabacein (1) with cucurbitacin B (2) was effected by acetylation of each to a common product, 3. The triacetate 3 was obtained in a chromatographically homogeneous but amorphous form; the identity of the samples obtained from the respective precursors 1 and 2 was established by ir, uv, nmr, mass spectrum, mixed tlc, and optical rotation comparisons.

The synthesis of fabacein (1) from cucurbitacin B (2) was effected *via* selective base-catalyzed solvolysis of the C-2 acetate group of the triacetate 3. Earlier studies in this laboratory have demonstrated a facilitation of the base-catalyzed solvolysis of the acetate esters of alcohols which bear carbonyl or hemiketal functions within hydrogen-bonding distance.[9] Accordingly, it was postulated that the alkaline solvolysis of the 2-acetate ester might be facilitated by the adjacent carbonyl group, possibly through hydrogen bonding of the acidic hydroxyl group of its hemiketal adduct with the carbonyl oxygen of the 2-acetate, as shown. In the event, treatment of 3 with triethylamine in 10% aqueous methanol for 12 hr at room temperature effected a smooth, selective solvolysis of the 2-acetate ester group, to yield fabacein (1).



(1) (a) Tumor Inhibitors. LXXXI. Part LXXX: S. M. Kupchan, R. W. Britton, M. F. Ziegler, C. J. Gilmore, R. J. Restivo, and R. F. Bryan, *J. Amer. Chem. Soc.*, **95**, 1335 (1973). (b) This investigation was supported by grants from the National Cancer Institute (CA-11718) and the American Cancer Society (T-275). (c) National Institutes of Health Postdoctoral Fellow, 1972–.

(2) W. O. Eisenhut and C. R. Noller, *J. Org. Chem.*, **23**, 1984 (1958).

(3) W. Schlegel and C. R. Noller, *ibid.*, **26**, 1211 (1961).

(4) We thank Professor C. R. Noller cordially for a generous sample of fabacein.

(5) S. M. Kupchan, A. H. Gray, and M. D. Grove, *J. Med. Chem.*, **10**, 337 (1967).

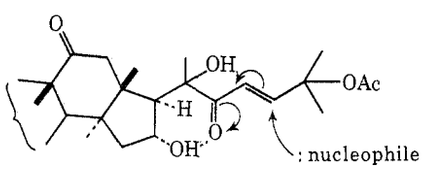
(6) S. M. Kupchan, R. M. Smith, Y. Aynehchi, and M. Maruyama, *J. Org. Chem.*, **35**, 2891 (1970).

(7) S. M. Kupchan, C. W. Sigel, L. J. Guttman, R. J. Restivo, and R. F. Bryan, *J. Amer. Chem. Soc.*, **94**, 1353 (1972).

(8) D. Lavie, Y. Shvo, O. Gottlieb, and E. Glotter, *J. Org. Chem.*, **28**, 1790 (1963).

(9) S. M. Kupchan, S. P. Eriksen, and M. Friedman, *J. Amer. Chem. Soc.*, **83**, 343 (1966).

Fabacein showed relatively low cytotoxicity (ED_{50} = 1 μg/ml) toward human carcinoma of the nasopharynx in tissue culture (KB),[10] in contrast with the potent cytotoxicity shown by cucurbitacin B ($ED_{50} \cong 10^{-6}$ μg/ml).[5] Earlier studies in this laboratory have demonstrated the importance of highly electrophilic conjugated systems in relation to the cytotoxicity of several classes of terpenoids.[11] Saturation of the conjugated Δ^{23} double bond in the cucurbitacins is accompanied by a profound lessening in cytotoxicity of the resultant dihydrocucurbitacin derivatives.[5,6] Consequently, reactions of the side chain conjugated ketone with biological macromolecules may play an important role in the mechanism by which cucurbitacins exert their cytotoxic effects. The marked diminution in cytotoxicity which accompanies the acetylation of the C-16 hydroxyl group of cucurbitacin B suggests that the free hydroxyl group may be important for the reactivity of the conjugated ketone. Thus, hydrogen-bonding interaction between the C-16 hydroxyl group and the C-22 ketone could activate the α,β-unsaturated ketone toward nucleophilic attack by a biological macromolecule, as shown. The lessened cytotoxicity



of fabacein, then, may result from the diminished reactivity of the conjugated ketone in the C-16 acetate ester.

Experimental Section

Melting points were determined on a Mettler FP2 melting point apparatus. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Ultraviolet spectra were recorded on a Coleman Hitachi EPS-3T recording spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Associates HA-100 spectrometer using TMS as an internal standard. Mass spectra were recorded on either Hitachi Perkin-Elmer RMU-63 or AEI MS-902 spectrometers, equipped with direct insertion probes. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Acetylation of Fabacein to Triacetate 3.—A solution of fabacein[4] (1, 12 mg) in anhydrous pyridine (0.5 ml) was treated with acetic anhydride (0.5 ml). The reaction mixture was stirred overnight at room temperature under nitrogen. The solution was evaporated *in vacuo* and the residue was dissolved in ethanol and reevaporated. The oily residue (12 mg) was separated by preparative tlc on Brinkmann Silplates with 1% methanol–chloroform. The major band was eluted with ethyl acetate. Attempts to crystallize the product were unsuccessful. The amorphous product (**3**, 10 mg) showed R_f 0.68 on Brinkmann Silplates with 2% methanol–chloroform; R_f 0.80 on ChromAr plates with 1% methanol–ether; R_f 0.70 on polyamide plates with 70% methanol–water; uv ($CHCl_3$) 230 nm (ε 10,000); $[\alpha]^{25}D$ +2.5° (*c* 3.60, $CHCl_3$); ir ($CHCl_3$) 2.92, 3.36, 3.43, 5.76, 5.92, 6.15, 7.38, 8.09, and 9.70 μ; nmr ($CDCl_3$) τ 2.94 (1 H, d, J = 16 Hz), 3.66 (1 H, d, J = 16 Hz), 4.32 (1 H, m), 4.62 (1 H, d of d, J = 14, 5 Hz), 4.90 (1 H, b t, J = 8 Hz), 5.80 (1 H, s), 7.92 (3 H, s), 8.04 (3 H, s), 8.19 (3 H, s), 8.46 (6 H, s), 8.62 (3 H, s), 8.72 (3 H, s), 8.94 (3 H, s), and 9.01 (3 H, s); mass spectrum *m/e* 582, 412, 385, 325, 189, 112, 111, 96, and 43.

Acetylation of Cucurbitacin B to Triacetate 3.—Cucurbitacin B (**2**, 40 mg) was acetylated as above. The product obtained after preparative tlc (35 mg) showed the same rotation, ir, uv, nmr, mass spectrum, and R_f values as the product (**3**) of acetylation of fabacein (**1**).

Solvolysis of Triacetate 3 to Fabacein (1).—A solution of triacetate **3** (30 mg) in 10% aqueous methanol (0.5 ml) was treated with triethylamine (4 drops) and allowed to stand overnight at room temperature. The solution was evaporated *in vacuo*. The major component (21 mg), obtained by preparative tlc on Brinkmann Silplates with 2% methanol–chloroform, was crystallized from dichloromethane–absolute ethanol. The product (10 mg), mp 197–200°, was characterized as fabacein (**1**) by mixture melting point, ir, uv, nmr, mass spectrum, and tlc comparisons with an authentic sample.

Registry No.—**1**, 37710-13-7; **2**, 6199-67-3; **3**, 37710-14-8.

(10) Cytotoxicity was assayed, under the auspices of the National Cancer Institute, by the procedure described in *Cancer Chemother. Rep.*, **25**, 1 (1962).

(11) S. M. Kupchan, *Pure Appl. Chem.*, **21**, 227 (1970).

Benzo[*b*]thiophenes from Thiophenes. A Facile Approach

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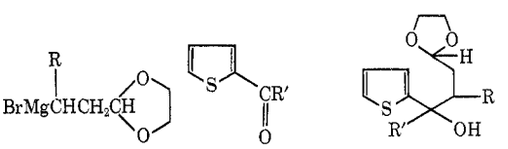
Benzo[*b*]thiophenes are generally synthesized by ultimate construction of the thiophene component onto thiophenol precursors. The alternative approach, *i.e.*, annellation of the benzene ring on preformed thiophenes, appears to have received scant attention.[1] Elaboration of such a route is herein described and is exemplified by the preparation of compounds **4a–d**, **4f–h**, and **5**.

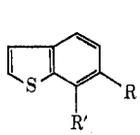
Grignard reagent **1a** has recently attracted attention



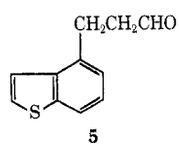
1a, R = H
b, R = CH_3

2a, R′ = H
b, R′ = CH_3
c, R′ = C_2H_5
d, R′ = *i*-C_3H_7
e, R′ = *t*-C_4H_9
f, R′ = OC_2H_5

3a, R = R′ = H
b, R = H; R′ = CH_3
c, R = H; R′ = C_2H_5
d, R = H; R′ = *i*-C_3H_7
e, R = H; R′ = *t*-C_4H_9
f, R = R′ = CH_3
g, R = CH_3; R′ = C_2H_5
h, R = H; R′ = $CH_2CH_2CHOCH_2CH_2O$



4a, R = R′ = H
b, R = H; R′ = CH_3
c, R = H; R′ = C_2H_5
d, R = H; R′ = *i*-C_3H_7
f, R = R′ = CH_3
g, R = CH_3; R′ = C_2H_5
h, R = H; R′ = CH_2CH_2CHO



5

as a synthetic tool.[2] We chose to treat it with thiophenes **2a–e**. Products **3a–d** were subsequently submitted to the action of 10% refluxing H_2SO_4; this brought about hydrolysis, cyclization, and aromatization and produced benzo[*b*]thiophenes **4a–d** in 60–70%

(1) B. Iddon and R. M. Scrowston in "Advances in Heterocyclic Chemistry," Vol. 11, A. R. Katritzky and A. J. Boulton, Ed., Academic Press, New York, N. Y., 1970, pp 177–381.

(2) G. Buchi and H. Wuest, *J. Org. Chem.*, **34**, 1121 (1969).