Psoralen Synthesis. Improvements in Furano Ring Formation. Application to the Synthesis of 4,5′,8-Trimethylpsoralen

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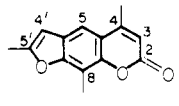
Two methods have been studied in depth for the linear fusion of a furano ring to a coumarin, as applied to the synthesis of 4,5′,8-trimethylpsoralen. Thus alkylation of 7-hydroxy-4,8-dimethylcoumarin (**9**) with 2,3-dichloro-1-propene followed by Claisen rearrangement and treatment of the rearrangement product with 70% sulfuric acid produces 4,5′,8-trimethylpsoralen (**1**) in 70% overall yield from **9**. At higher acid concentrations, a dimer of **1** is a major product. Alternatively, amination of hydroxycoumarin **9** by exchange with *O*-(2,4-dinitrophenyl)hydroxylamine proceeds in high yield to give the *O*-coumarylhydroxylamine **13**. The oxime **8**, formed by condensation of **13** with acetone, rearranges on treatment with acid to give trimethylpsoralen **1** along with **9**, the acetonyl ether of **9**, and a C-8 acetonyl derivative of **9**.

4,5′,8-Trimethylpsoralen (**1**)[2] and its derivatives **2** have drawn attention as effective photoreactive cross-linking reagents for nucleic acids.[3] As part of a detailed structure–activity study, we sought good and general methods for synthesizing 5′-methylpsoralens. Both as a model and because of its intrinsic importance, we focused this search on improved methods for making **1**.



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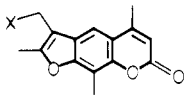
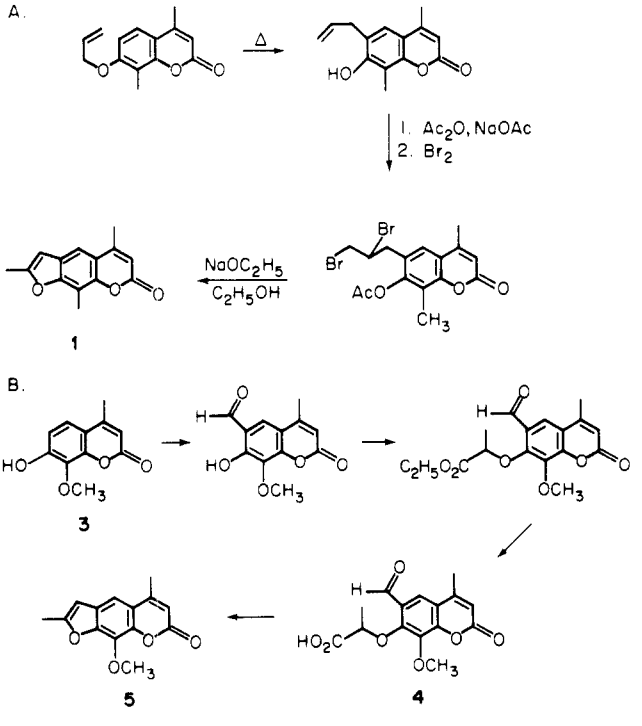


2, X = -OH, $-\overset{+}{N}H_3$

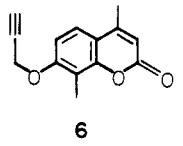
Two approaches have been reported for the synthesis of 5′-methylpsoralens. One approach utilized Claisen rearrangement of 7-allyloxycoumarins, illustrated by the synthesis of trimethylpsoralen **1** according to Scheme IA.[4] The other approach is illustrated in Scheme IB and involves synthesis and ring closure of propionic acid derivative **4**.[5] In the latter approach both the formylation of **3** with hexamethylenetetramine[6] and the ring-closure elimination–decarboxylation of **4** to **5** proceed poorly, giving **5** from **3** in 4% overall yield. In the former approach the last step requires use of alkali and proceeds only in moderate yield,[7] giving **1** in 28% overall yield from hydroxycoumarin **9**. Both of these approaches are extensions of methods used for the synthesis of 2-methylbenzofurans.

Scheme I. Syntheses of 5′-Methylpsoralens



There are many methods available for the synthesis of benzofurans,[8] but most methods are of limited scope, require many steps for precursor synthesis, and/or proceed in low yield. Among methods used for synthesis of 2-methylbenzofurans, three appeared attractive in that they proceed from the corresponding phenols in at least good overall yield, and extension to the synthesis of psoralen **1** would require only two or three steps from the readily available hydroxycoumarin **9**. Two of these methods involve Claisen rearrangements, one of propargyl ether **6** and the other of β-chloroallyl ether **7**. The third method requires use of oxime **8** in a Fischer indole-like synthesis. In this report we describe syntheses of compounds **6**, **7**, and **8** and their conversion to trimethylpsoralen **1**.

Rearrangement of Coumaryl Propargyl Ether 6. Aryl propargyl ethers give 2-methylbenzofurans if heated in sulfolane in the presence of potassium carbonate.[9] Extension of this approach to synthesis of **1** required ether **6**, which was prepared in good yield from hydroxycoumarin **9**[10] by alkylation with propargyl bromide. However, treatment of **6** according to the reported procedure[9,11] led to formation of intractable material, from which **1** was isolated in only about 2% yield. Numerous variations in the procedure[11] did not lead to any improvement.



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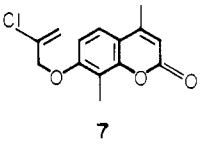

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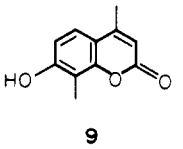

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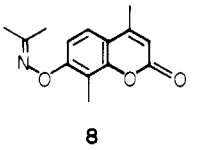

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Preparation of 1 via β-Chloroallyl Ether 7. Heating β-chloroallyl phenyl ether gives the expected product of Claisen rearrangement along with 2-methylbenzofuran.[12] The Claisen rearrangement product and analogous compounds have been converted to 2-methylbenzofurans or to 2-methylfurans by heating in the presence of alkali,[13] by treating with concentrated sulfuric acid[14] or with 90% sulfuric acid,[15] and by heating in concentrated hydrochloric acid.[16] Yields in these

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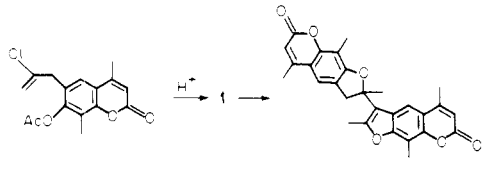
examples vary from poor to good, depending on both substituents and conditions of ring closure.

Extension of this method to the synthesis of **1** required ether **7**, which was prepared in high yield by alkylation of phenol **9** with 2,3-dichloro-1-propene in dimethylformamide (DMF)/benzene in the presence of potassium carbonate and a catalytic amount of potassium iodide. Alkylation in acetone or in methyl ethyl ketone took place poorly. Rearrangement of chloroallyl ether **7** to **10** was accomplished in high yield by heating **7** in *p*-diisopropylbenzene/acetic anhydride at 200 °C. Subsequent ring-closure elimination of **10** to the psoralen **1** by heating in concentrated hydrochloric acid was ineffective due to lack of solubility. Addition of dioxane as cosolvent allowed for a one-phase reaction, but the yield of **1** was only moderate, and the crude product proved difficult to purify.

Treatment of **10** with concentrated sulfuric acid at room temperature led to its rapid consumption, but no product could be isolated. However, treatment with 90% sulfuric acid at 0 °C allowed for isolation of two products, one of which was psoralen **1** and the other was shown to be dimer **11**. If reaction in 90% sulfuric acid was allowed to continue for more than 1.5 h, both **1** and **11** were no longer present. Psoralen **1** in 90% sulfuric acid gave identical results, thus confirming that dimer **11** is formed from **1**. In the presence of 80% sulfuric acid at 0



10 **11**

°C, **10** produced a mixture in which the amount of **1** relative to **11** was increased, and use of 70% sulfuric acid at room temperature led to isolation of pure **1** in 80% yield.

The structure of dimer **11** was suggested by the formation of analogous dimers from benzofurans under acidic conditions.[17] Its NMR spectrum showed absorptions expected for the aryl protons and aryl methyl groups; in addition there was an upfield singlet at δ 1.87 consistent with the remaining methyl group and a broad singlet at δ 3.60 consistent with the methylene group. Low and high resolution mass spectral data confirmed the structure of dimer **11**.

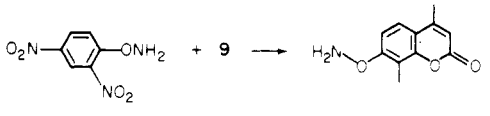
Conversion of coumarin **9** to trimethylpsoralen **1** does not require extensive purification of intermediates **7** and **10**, although chromatography of **7** and **10** is recommended before using them in subsequent reactions. Carried out in this manner, an overall (**9** → **1**) yield of 70% can be obtained. Conversion of **9** to **1** as described in Scheme IA gives **1** in an overall yield of 28%.[4]

Synthesis and Rearrangement of *O*-Coumaryloxime 8. The Fischer indole synthesis has been extended to the synthesis of benzofurans.[18] Although many benzofurans have been prepared by this method,[8] all fall within a few structural types. This limitation stems from the methods available for synthesis of the precursor *O*-aryloximes.

Two methods have been used for the synthesis of *O*-aryloximes. One method utilizes condensation of an *O*-arylhydroxylamine with a carbonyl compound, and the other method involves attack by the anion of an oxime on an aryl group containing a suitable leaving group (usually halide) and activated by one or more ortho and para electron withdrawing groups. The latter approach is clearly of limited scope, and the former approach involves (1) *O*-arylhydroxylamines, made by displacement reactions analogous to the latter approach,[19] or (2) *O*-phenylhydroxylamine, which in turn is prepared in poor yield from potassium phenoxide and hydroxylamine-*O*-sulfonic acid[20] or from diphenyliodonium bromide.[21]

Clearly the rearrangement of *O*-aryloximes has potential as a general method of benzofuran synthesis if one had available a high-yield, convenient synthesis of *O*-arylhydroxylamines directly from the corresponding phenol. Such a synthesis appeared possible if one could effect amination by exchange with *O*-(2,4-dinitrophenyl)hydroxylamine (**12**),[22,23] which contains a good leaving group and is stable at room temperature. Accordingly, we attempted synthesis of *O*-coumarylhydroxylamine **13** from **12** and hydroxycoumarin **9**.

Amination was carried out by preforming the anion of **9** with potassium or sodium hydride in DMF followed by addition of **12**. If **12** and **9** were used in a 1/1 mol ratio, **13** could be obtained in 40% yield, but better results were obtained by using excess hydroxycoumarin **9**. With **12** and **9** in a mole ratio of 1/2, consumption of **12** was complete at room temperature

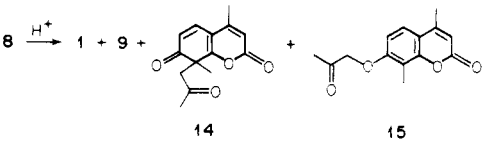


12 **13**

in less than 3 h, and **13** was formed in 70% yield based on aminating reagent. Unreacted **9** could be recovered quantitatively. Conversion of hydroxylamine **13** to oxime **8** took place rapidly in 89% yield in the presence of a catalytic amount of concentrated hydrochloric acid.

A wide variety of catalysts have been used for the rearrangement step of the Fischer indole synthesis,[24] and a limited number of these catalysts have been reported to effect conversion of *O*-aryloximes to benzofurans. Thermal rearrangement of *O*-aryloximes has been attempted in a few cases, but only intractable tars were obtained.[25] In the case of oxime **8** nearly every attempt at rearrangement led to formation of at least minor amounts of hydroxycoumarin **9**.[26] Use of acetic acid, concentrated hydrochloric acid in ethanol, zinc chloride in acetic acid or in ethanol, and heat alone led to formation of **9** as the major product. With boron trifluoride etherate in acetic acid many products were formed, including minor amounts of **1**, while use of sulfuric acid in acetic acid and of poly(phosphoric acid) led to formation of fewer products but still produced **1** only in poor yield. Use of phosphorus pentoxide in methanesulfonic acid[27] gave none of **1**.

Formation of **1** in moderate yield was accomplished when **8** was heated at 60 °C in the presence of phosphoric acid in acetic acid or in formic acid, and in formic acid alone. In the case of phosphoric acid in formic acid, all significant products were isolated. In addition to **1** (40% yield) and minor amounts of **9**, **14** and **15** were also formed in 17 and 5% yields, respec-

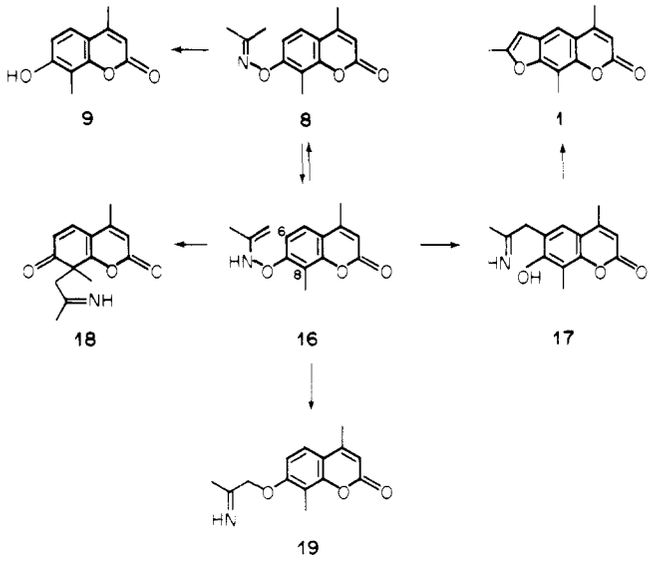


14 **15**

tively. The NMR spectrum of **14** exhibited consistent absorptions for the C-4 and acetonyl methyl groups and for the protons at C-3, C-5, and C-6. In addition, the spectrum contained an upfield singlet consistent with the C-8 methyl group and a broad singlet at δ 3.53 to which is assigned the methylene of the acetonyl group. IR and both low- and high-resolution mass spectral data confirmed the structure **14**. The structure of **15** was confirmed by synthesis from **9** and chloroacetone.[28]

Products **1**, **14**, and **15** presumably arose as outlined in Scheme II. Tautomerization of **8** would give rise to eneamine **16**, the structural type analogous to the key intermediate in the Fischer indole synthesis[24] and in the rearrangement of *O*-aryloximes.[29] Rearrangement of the eneamine portion to the open C-6 position would lead to imino compound **17**, while

Scheme II. Proposed Reaction Paths for O-Coumaryloxime 8



rearrangement to C-8 would give imino ketone **18**. Ring closure of **17** followed by loss of ammonia would lead to furocoumarin **1**, but in the case of **18** a similar path is not available, thus allowing for hydrolysis to **14** in the reaction solution.

Formation of compounds (β-ketosulfides) analogous to **15** has been observed during attempts to rearrange *S*-arylthiooximes to benzo[*b*]thiophenes.[18c,30] There is little doubt that **15** arose from hydrolysis of **19**, but the mechanism of formation of **19** is open to question. One can write a unimolecular mechanism involving rearrangement of **16** directly to **19**, but studies of the mechanism of the rearrangement of *S*-arylthiooximes suggest an alternative mechanism beginning with cleavage of the N–O bond of **8** by nucleophilic attack on oxygen.[30]

In summary, a highly efficient synthesis of 5′-methylpsoralens, and specifically 4,5′,8-trimethylpsoralen, has been developed via the chloro allyl ether of **9**. Also, the rearrangement of *O*-coumaryloximes appears attractive as an alternative method of potential broad scope.

Experimental Section[31]

4,8-Dimethyl-7-propargyloxycoumarin (6). 4,8-Dimethyl-7-hydroxycoumarin (**9**)[10] (2.28 g, 12 mmol), propargyl bromide (1.43 g, 12 mmol), K_2CO_3 (2.14 g, 15.5 mmol), and acetone (6.0 mL) were combined and stirred at 70 °C for 7 h. The reaction mixture was evaporated, and the residue was taken up in water (100 mL) and ether (700 mL), washing the organic layer with 1 M NaOH (75 mL), water (75 mL), and then saturated NaCl (2 × 75 mL), then drying, and evaporating. The residue was rinsed with petroleum ether to remove residual propargyl bromide, then evacuated at 0.02 mm to give a residue (2.28 g) which was recrystallized from $CHCl_3$/hexanes: yield 2.06 g (75%); mp 141–143 °C; NMR δ 2.30 (3 H, s), 2.38 (3 H, d, J = 2 Hz), 2.55 (1 H, t, J = 2.5 Hz), 4.82 (2 H, d, J = 2.5 Hz), 6.12 (1 H, m), 6.96 (1 H, d, J = 9 Hz), 7.44 (1 H, d, J = 9 Hz).

Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.7; H, 5.3. Found: C, 73.7; H, 5.3.

Rearrangement of 6. Ether **6** (1.14 g, 5.0 mmol), sulfolane (10 mL, Aldrich 99%, held over 4A sieves at 40–45 °C for 16 h, then distilled, bp 90 °C (0.12 mm)), and K_2CO_3 (0.69 g, 5.0 mmol) were heated for 3 h with vigorous mechanical stirring at 220 °C (T_b). The dark mixture was cooled to room temperature and water (30 mL) was added with vigorous stirring, resulting in formation of a large amount of tarry material. This mixture was extracted with $CHCl_3$ (2 × 30 mL), resulting in formation of emulsions which required one to several hours to settle. The combined extracts were dried and evaporated to remove chloroform, and most of the sulfolane was removed by bulb-to-bulb distillation (90–100 °C (0.1 mm)), leaving a residue (1.46 g). Chromatography (twice) of this residue on silica gel (3–5 g) with chloroform removed some of the dark material, and a second bulb-to-bulb distillation removed more sulfolane, now leaving a residue of 0.58 g which was sublimed (0.7 mm, 110 °C, 15 h). Chromatography (silica, 5 g, $CHCl_3$) of the sublimate and recrystallization ($CHCl_3$/EtOAc) yielded 18 mg (2%) of **1**; the mother liquors contained more of **1** as indicated by TLC and NMR.

7-(β-Chloroallyloxy)-4,8-dimethylcoumarin (7). A. 4,8-Dimethyl-7-hydroxycoumarin (**9**, 19.0 g, 0.10 mol) and DMF (143 mg, dried over 4A sieves) were heated at 70 °C (T_b) with mechanical stirring until **9** dissolved; then benzene (143 mL), K_2CO_3 (18.7 g, 0.13 mol), KI (0.86 g, 5 mmol) and 2,3-dichloro-1-propene (13.1 g, 0.118 mol, bp 92–93 °C) were added in the order given. This mixture was mechanically stirred at 80–85 °C (T_b) for 11 h, cooled, and evaporated at 15 mm to remove benzene, then at 0.1 mm to remove DMF. The residue was diluted with $CHCl_3$ (400 mL) and washed with water (500 mL). The aqueous layer was extracted further with $CHCl_3$ (100 mL), and the combined extracts were washed with 1 M NaOH (400 mL) and saturated NaCl (400 mL) and dried. Evaporation gave 26.3 g (99%) of solid, giving on TLC (CH_2Cl_2) one spot corresponding in R_f with **7** obtained as described in B, below.

B. Coumarin **9** (1.33 g, 7 mmol) was converted to crude **7** as described in A. Chromatography on silica gel (15 g) with $CHCl_3$ yielded a residue (1.82 g, 98%) which was recrystallized from $CHCl_3$/hexanes to give after collection of three crops 1.67 g (90%) of **7**: mp 117–118 °C; NMR δ 2.37 (3 H, s), 2.38 (3 H, d, J = 2 Hz), 4.68 (2 H, m), 5.55 (2 H, m, J_{gem} = 7 Hz), 6.14 (1 H, m), 6.82 (1 H, d, J = 9 Hz), 7.44 (1 H, d, J = 9 Hz); UV (95% C_2H_5OH) λ_{max} 244 nm (ϵ 3920), 254 (4120), 319 (14 600).

Anal. Calcd for $C_{14}H_{13}O_3Cl$: C, 63.5; H, 5.0. Found: C, 63.7; H, 5.1.

7-Acetoxy-6-(β-chloroallyl)-4,8-dimethylcoumarin (10). Ether **7** (264 mg, 1.0 mmol), *p*-diisopropylbenzene (4 mL, refluxed over Na for 16 h, then distilled from Na) and acetic anhydride (0.4 mL) were refluxed (T_i 195 °C) under argon for 26 h. The cooled reaction mixture was diluted with $CHCl_3$ (5 mL), washed with water (10 mL) and then saturated $NaHCO_3$ (10 mL), dried, and evaporated at 15 mm to remove $CHCl_3$, then at 50 °C (0.1 mm) to remove *p*-diisopropylbenzene. The residue was chromatographed on silica gel (2.8 g) eluting with CH_2Cl_2 to yield a residue (300 mg) which was recrystallized from $CHCl_3$/hexanes to give **10** (185 mg, 60%): mp 161–162 °C; NMR δ 2.27 (3 H, s), 2.38 (3 H, s), 2.42 (3 H, d, J = 2 Hz), 3.64 (2 H, broad s), 5.25 (2 H, m, J_{gem} = 11 Hz), 6.28 (1 H, m), 7.40 (1 H, broad s); UV (95% C_2H_5OH) λ_{max} 244 nm (sh, ϵ 7090), 278 (11 800), 315 (6900).

Anal. Calcd for $C_{16}H_{15}O_4Cl$: C, 62.7; H, 4.9. Found: C, 62.6; H, 5.0.

Examination of an NMR spectrum of the mother liquors indicated the presence of **10** and **1** in a mole ratio of 9/1.

4,5′,8-Trimethylpsoralen (1) from 10. Coumarin **10** (7.47 g, 24.4 mmol) was shaken on a mechanical shaker with 70% (v/v) sulfuric acid (171 mL of concentrated H_2SO_4 plus 74 mL of water) for 1.0 h. The mixture was then added to water (1.72 L) with vigorous mechanical stirring at a rate which allowed maintainance of T_i at 10–20 °C. The mixture was then extracted with chloroform (2 × 500 mL). The combined extracts were washed with saturated $NaHCO_3$ (600 mL), cooled to 5 °C, washed with cold 1 M NaOH (400 mL) and saturated $NaHCO_3$ (400 mL), dried and evaporated to a residue which was recrystallized from $CHCl_3$/ethyl acetate to yield **1** (4.48 g, 80%): mp 231–232 °C (lit.[4] mp 234–235 °C; mp of material purchased from the Paul B. Elder Co., 230–231 °C); identical by TLC, NMR, and UV comparison with authentic material; NMR δ 2.52 (6 H, m), 2.57 (3 H, s), 6.20 (1 H, m), 6.40 (1 H, m), 7.50 (1 H, broad s).

Conversion of 9 to 1. Coumarin **9** (4.75 g, 25 mmol) was converted to **7** as described in part A above, yielding a crude residue (6.53 g, 99%) which was chromatographed on silica gel (30 g) with chloroform to yield a residue (**7**, 6.47 g, 98%), giving a single spot on TLC ($CHCl_3$ or CH_2Cl_2). This residue was combined with *p*-diisopropylbenzene (110 mL, prepared as described above) and acetic anhydride (3.84 g) and refluxed (T_i 200 °C) under argon for 63 h (after 42 h and again after 50 h, 0.1 mL of acetic anhydride was added as called for by the presence of a low R_f spot due to unacetylated **10** on TLC of evaporated aliquots). The solution was cooled and the resulting mixture was diluted with $CHCl_3$ (300 mL), washed with water (200 mL) and saturated $NaHCO_3$ (200 mL), dried, and evaporated to a residue (7.61 g) which was chromatographed on silica gel (30 g) with CH_2Cl_2 to yield a residue (7.23 g of **10**) which was powdered before being converted to **1** as described above. The yield of recrystallized **1** was 3.78 g, 70%, 66% overall from **9**, and after chromatography (5 g silica with $CHCl_3$) and recrystallization a second crop (0.40 g, 7% overall from **9**) was obtained from the mother liquors. Chromatography of the mother liquors from the second crop on Kieselgel (22 g) with $CHCl_3$ yielded dimer **11** (0.17 g, 3%) and a mixture containing **1** (0.1 g) and **10** (0.2 g).

TLC (silica/$CHCl_3$) of trimethylpsoralen **1** obtained by this procedure indicated the presence of a small amount of a contaminant with

R_f lower than that of 1. The combined crops were recrystallized ($CHCl_3$/ethyl acetate) to give **1** (3.70 g, 65% overall from **9**) which was shown to be free of this contaminant by TLC and by LC (Spherisorb ODS, 5 μm in a 4.6 × 250 mm column) eluting with CH_3OH/H_2O (70/30, v/v).

Dimer 11. To 90% (v/v) sulfuric acid (4 mL) at 0–5 °C (T_i) was added with magnetic stirring compound **10** (100 mg, 0.32 mmol) over 1.5 min. After 10 min, the solution was added dropwise over 1 min to 30 mL of water with rapid stirring and cooling. The mixture was extracted with $CHCl_3$ (2 × 10 mL), and the combined extracts were dried and evaporated to yield a residue (56 mg) which was chromatographed on Kieselgel (6 g) with $CHCl_3$ to yield **1** (18 mg) and dimer **11** (29 mg): NMR δ 1.87 (3 H, s), 2.32 (3 H, d, J = 2 Hz), 2.38 (3 H, s), 2.42 (3 H, d, J = 2 Hz), 2.52 (3 H, s), 2.58 (3 H, s), 3.60 (2 H, m), 6.02 (1 H, m), 6.15 (1 H, m), 7.22 (1 H, s), 7.55 (1 H, s); MS m/e (rel intensity) 457 (11), 456 (37, M^+), 228 (39), 44 (100); high-resolution mass spectrum, calcd for $C_{28}H_{24}O_6$ (M^+), 456.1573, found, 456.1558.

7-Aminoxy-4,8-dimethylcoumarin (13). A. Sodium hydride (220 mg, 50% NaH in oil dispersion) was diluted and stirred with dry hexane (6 mL). The mixture was allowed to settle, and the hexane was drawn off. This process was repeated twice, then the residue was dried by sweeping N_2 through the flask while stirring. To the residue (142 mg, 5.9 mmol of NaH) was added DMF (13 mL, distilled from CaH_2), and the mixture was cooled in an ice-water bath. Coumarin **9** (1.12 g, 5.9 mmol) in DMF (11 mL) was added dropwise over 5 min while maintaining T_i at 5–10 °C. The cooling bath was removed after 0.5 h and hydroxylamine **12**[22b] (588 mg, 2.95 mmol) in DMF (7 mL) was added over 3 min. The solution was stirred for 3 h and then added with stirring and cooling to a solution of water (175 mL) and saturated $NaHCO_3$ (42 mL) followed by extraction with $CHCl_3$ (1 × 175 mL plus 2 × 75 mL). The combined extracts were cooled to 5–10 °C and washed with cold 1 M NaOH (175 mL) and saturated $NaHCO_3$ (100 mL), dried, and evaporated to a residue (0.42 g, 69%) which was identical by TLC and spectral comparison with authentic material obtained as described below. The alkaline wash was neutralized (3.0 M H_3PO_4 to pH 7–8) with stirring and cooling and allowed to stand 24 h. The mixture was then filtered, and the residue was washed with cold water (15 mL) and dried to yield a residue (665 mg, 91%) identical with **9** by TLC and NMR.

B. Hydroxylamine **13** (64 mg), obtained as described above, was recrystallized from absolute ethanol to yield 50 mg of fine needles: mp 155–156 °C dec; NMR (Me_2SO-d_6) δ 2.20 (3 H, s), 2.42 (3 H, d, J = 2 Hz), 6.18 (1 H, m), 7.22 (2 H, br s), 7.44 (1 H, d, J = 9 Hz), 7.62 (1 H, d, J = 9 Hz); UV (95% C_2H_5OH) λ_{max} 247 nm (ε 3720), 256 (3690), 324 (16 200).

Anal. Calcd for $C_{11}H_{11}NO_3$: C, 64.4; H, 5.4; N, 6.8. Found: C, 64.1; H, 5.4; N, 7.1.

Acetone *O*-7-(4,8-Dimethylcoumaryl)oxime (8). A. Hydroxylamine **13** (205 mg, 1.0 mmol), absolute ethanol (10 mL), acetone (64 mg, 0.081 mL, 1.1 mmol), and concentrated HCl (2 drops) were combined in the order given and stirred. Within 5 min, this heterogeneous mixture had become homogeneous, and within another 10 min, it was heterogeneous. After 1 h, the mixture was evaporated to a residue (237 mg) which was chromatographed on silica gel (2 g) with CH_2Cl_2 to yield a residue which on recrystallization from absolute ethanol yielded 211 mg (86%) of **8**: mp 129–130 °C dec; NMR δ 2.08 (3 H, s), 2.15 (3 H, s), 2.33 (3 H, s), 2.40 (3 H, d, J = 2 Hz), 6.08 (1 H, m), 7.38 (2 H, s); UV (95% C_2H_5OH) λ_{max} 248 nm (ε 5880), 257 (5830), 323 (18 400).

Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.6; H, 6.2; N, 5.7. Found: C, 68.5; H, 6.2; N, 5.7.

B. Hydroxylamine **13** (420 mg, 2.05 mmol, obtained as described in A above), absolute ethanol (40 mL), acetone (238 mg, 4.10 mmol), and concentrated HCl (3 drops) were combined in the order given and stirred for 3 h. The reaction solution was evaporated and the residue was chromatographed on silica gel (3 g) with CH_2Cl_2 to yield a residue which on recrystallization from ethanol yielded 448 mg (80%) of **8**, identical in all respects with **8** obtained as in A.

Rearrangement of Oxime 8. 4,5′,8-Trimethylpsoralen (1) and Compounds 14 and 15. Oxime **8** (245 mg, 1.0 mmol) was dissolved in 88% formic acid (30 mL), then 85% H_3PO_4 (3.3 ml) was added, and the solution was stirred at 60 °C (T_b) for 4 h. The cooled reaction solution was added to cold water (200 mL) with stirring and cooling, and this solution was extracted with $CHCl_3$ (2 × 125 mL). The combined extracts were washed with saturated $NaHCO_3$ (100 mL), 1 M NaOH (100 mL), and again with saturated $NaHCO_3$ (75 mL), dried, and evaporated to a residue (188 mg, 82%) which was chromatographed on Kieselgel (20 g) with CH_2Cl_2 to separate trimethylpsoralen **1** (94 mg, 41%). The remaining material was chromatographed on Kieselgel (8 g) with $CHCl_3$ to yield **15** (12 mg) and **14** (37 mg).

Fraction containing **15**: identical by NMR and TLC with material prepared as described in the following section.

Fraction containing **14**: NMR δ 1.33 (3 H, s), 2.10 (3 H, s), 2.30 (3 H, d, J = 2 Hz), 3.53 (2 H, br s), 6.04 (1 H, m), 6.15 (1 H, d, J = 10 Hz), 7.45 (1 H, d, J = 10 Hz); IR ($CHCl_3$) 1672, 1727 (sh), 1742 cm^{-1}; MS m/e (rel intensity) 246 (M^+, 13), 203 (62), 189 (9), 175 (23), 43 (100); high-resolution mass spectrum, calcd for $C_{14}H_{14}O_4$, 246.0892, found, 246.0886.

7-Acetonyloxy-4,8-dimethylcoumarin (15). Coumarin **9** (1.9 g, 10 mmol) was dissolved in DMF (14.3 mL, distilled from CaH_2), benzene (14.3 mL, distilled from CaH_2), K_2CO_3 (1.87 g, 13 mmol), and chloroacetone (1.13 g, 12 mmol, freshly distilled) were added, and the mixture was stirred at 80 °C (T_b). After 1.2 h, more chloroacetone (0.2 mL) was added, and after another 1.5 h the mixture was cooled and the solvents were evaporated. The residue was taken up in $CHCl_3$ (60 mL) and water (60 mL), and the aqueous layer was again extracted with $CHCl_3$ (40 mL). The combined extracts were cooled and washed with cold 1 M NaOH (55 ml) and then saturated $NaHCO_3$ (50 mL), dried, and evaporated to a residue which was recrystallized from absolute ethanol: yield of **15**, 2.20 g (90%); mp 171–172 °C (lit.[28] mp 173 °C): NMR δ 2.33 (3 H, s), 2.38 (6 H, br s), 4.65 (2 H, s), 6.14 (1 H, m), 6.63 (1 H, d, J = 9 Hz), 7.38 (1 H, d, J = 9 Hz).

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Registry No.—**1**, 3902-71-4; **6**, 69897-62-7; **7**, 69897-63-8; **8**, 69897-64-9; **9**, 4115-76-8; **10**, 69897-65-0; **11**, 69897-66-1; **12**, 17508-17-7; **13**, 69897-67-2; **14**, 69897-68-3; **15**, 21861-38-1; propargyl bromide, 106-96-7; 2,3-dichloro-1-propene, 78-88-6; chloroacetone, 78-95-5.

References and Notes

(1) Fellow of The Jane Coffin Childs Memorial Fund for Medical Research.
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Antitumor Agents. 33. Isolation and Structural Elucidation of Bruceoside-A and -B, Novel Antileukemic Quassinoid Glycosides, and Brucein-D and -E from *Brucea javanica*[1]

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The active extract of the seeds of *Brucea javanica* has yielded bruceoside-A (**1**) and -B (**2**), two novel potent antileukemic quassinoid glycosides of bruceosin (**15**) and brusatol (**8**), respectively, as well as brucein-D (**3**) and -E (**4**). The structure and stereochemistry of these compounds have been established from chemical transformation, correlations, and spectral analyses.

The seeds of *Brucea javanica* (Linn.) Merr (Simaroubaceae) are known as "Ya-Tan-Tzu" in Chinese folklore and as herbal remedies for human amebiasis, as well as for cancer.[2-4] As a result of the continuing search among plants for new and novel naturally occurring potential antitumor agents, the methanolic extract of the seeds of *B. javanica* was found to show significant inhibitory activity in vivo against the Walker 256 carcinosarcoma in rats and the P-388 lymphocytic leukemia in the mouse.[5] A preliminary communication[6] described the structural determination of the potent novel antileukemic principle, bruceoside-A (**1**). The purpose of this paper is to present the full account of the isolation and structural elucidation of bruceoside-A (**1**) and bruceoside-B (**2**), the new antileukemic substance, as well as the companion quassinoids brucein-D (**3**) and brucein-E (**4**). Bruceoside-A and -B appear to be the first two quassinoid glucosides which have been demonstrated to have antileukemic activity.[6] Brucein-D demonstrated significant antisarcoma activity in the Walker 256 screen.[6] The seeds of *B. javanica* were first defatted by *n*-hexane, and the marc was then extracted with methanol. The active methanol extract was concentrated and partitioned between chloroform and water (1:1). Guided by the in vivo P-388 assay, the active principles were concentrated in both the chlorofrom extract as well as in the water extract. The active water extract was further extracted with saturated butanol–water. Chromatography of the active butanol extract over silica gel led to the isolation of the active principles, bruceoside-A and -B and brucein-D and -E. Isolation of the active principles from the chlorofrom extract is in progress.

Bruceoside-A (**1**), $C_{32}H_{42}O_{16}$, showed the presence of hydroxyl, conjugated enone, and lactone moieties in its IR spectrum. Its NMR spectrum revealed the presence of a senecioyl group at δ 1.93, 2.16 (CH_3-23), and 5.36 (H-22), a carbomethoxyl group (δ 3.76), an angular methyl group (δ 1.60), a secondary methyl group [δ 1.15 (d, J = 6.0 Hz)], and low-field protons at δ 6.02 (H-15) and 6.84 (H-1) as a doublet and a singlet, respectively (Table I).

Acid hydrolysis of **1** with 3 N sulfuric acid–methanol (1:1) yielded D-glucose, identified by GLC as its trimethylsilyl derivative, and the major aglycon, which was identified as brusatol (**8**).[8] Structural characterization of **8** was based upon spectral evidence described before[6] as well as decoupling experiments (100 MHz, $CDCl_3$). For example (Table I), irradiation of H-14 (dd, J = 1.0 and 13.0 Hz) at δ 3.12 collapsed the H-15 doublet (J = 13.0 Hz) to a singlet. Conversely, irradiation of H-15 converted H-14 to a singlet. Irradiation of H-22 (m) at δ 5.64 changed the Me_2-23 doublets (J = 1.5 Hz each) at either δ 1.93 or 2.20 to a singlet. Conversely, irradiation of Me_2-23 at either δ 1.93 or 2.20 converted the H-22 multiplet to a doublet (J = 1.5 Hz). Final identification of **8** was established by a direct comparison with an authentic sample of brusatol, isolated from *Brucea sumatrana* by Geissman and co-workers.[8] As is apparent from the ^1H NMR data given in Table I, compound **8** lacks the characteristic signals of H-1 and Me-4 as found in **1** and shows the presence of a C-4 vinyl methyl group as a doublet (J = 2.0 Hz) at δ 1.84. Consequently, it was concluded that compound **8** was a secondary product formed during the acid hydrolysis of **1**. En-

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