is sexually linked in this population, this system appears promising for further explorations of genetical, physiological, ecological and evolutionary aspects of migratory behavior. It should be noted that qualitative sexual differences in migratory behavior have been detected in a partial migrant population of blackcaps as well. Males from southern France were significantly less likely to show any migratory behavior at all relative to females from the same population [16]. It seems reasonable to suggest that sexual differences in endogenously programmed migratory activity in autumn have ultimate roots in intraspecific competition during the nonreproductive season although other factors may certainly be involved. With respect to differential migratory activity in spring, males of most migrant species precede females in arriving at the breeding grounds, presumably to establish territories at the earliest possible time. In blackcaps, males precede females by about a week [12], a pattern that matches closely the differential migratory activity displayed by our experimental birds.

Results in this direction have also been found in an another annual migrant, the dark-eyed junco (*Junco hyemalis*), but the tendency toward differential migratory activity between the sexes under laboratory conditions was generally insignificant [17]. Juncos show a pronounced bias for females to migrate farther than males [17]. So, in combination, the laboratory and field studies indirectly implicate a relatively strong role for environmental factors in regulating sexual differential migration in this species. Recent experiments on juncos appear to support this possibility [18].

Although these results indicate that an endogenous component could, in theory, be entirely responsible for differential migration in this population of blackcaps, they do not preclude the possibility that exogenous factors might also play a potentially important proximate role. Further experimental work in this direction is currently being undertaken.

1 This research was supported by the Alexander von Humboldt Foundation (Terrill) and the Deutsche Forschungsgemeinschaft (Berthold).
2 Gauthreaux, S. A. Jr, in: Avian Biology VI, p. 93. Eds D. S. Farner, J. R. King and K. C. Parkes. Academic Press, New York 1982.
3 Terrill, S. B., Proc. XIX Internat. Orn. Congr. Ottawa (1988) in press.
4 Terrill, S. B., Studies in Avian Biology. (1988) in press.
5 Kennedy, J. S., in: Migration: Mechanisms and Adaptive Significance, p. 5. Univ. Texas, Austin 1985.
6 Berthold, P., J. evol. Biol. *1* (1988) 195.
7 Gwinner, E., Adv. Study Behav. *16* (1986) 191.
8 Sutherland, W. J., Nature *334* (1988) 471.
9 Terrill, S. B., and Able, K. P., Auk *105* (1988) 205.
10 Berthold, P., et al., unpublished data.
11 Olioso, G., Bievre *8* (1985) 63.
12 Sharrock, J. T. R., The Atlas of Breeding Birds in Britain and Ireland, p. 368. T. and A. D. Poyser Ltd., Carlton, Great Britain.
13 Biebach, H., Auk *100* (1983) 601.
14 Berthold, P., Ring *10* (1984) 253.
15 Lundberg, P., J. theor. Biol. *125* (1987) 351.
16 Berthold, P., Ric. Biol. Selvaggina *10* (1986) 33.
17 Ketterson, E. D., and Nolan, V. Jr, in: Migration: Mechanisms and Adaptive Significance, p. 553. Ed. M. A. Rankin. Univ. Texas, Austin 1985.
18 Terrill, S. B., Behav. Ecol. Sociobiol. *21* (1987) 1.

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Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4 [1]

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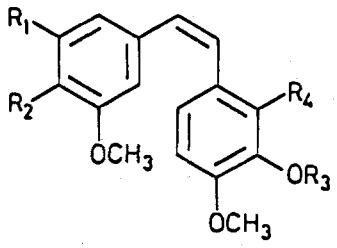
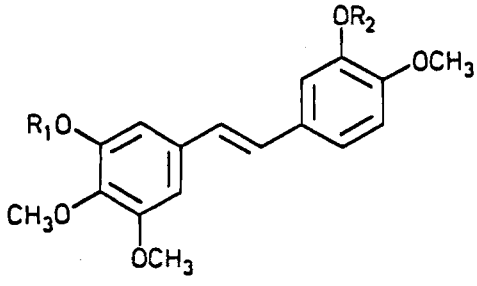
Summary. The African tree *Combretum caffrum* (Combretaceae) has been found to contain a powerful inhibitor of tubulin polymerization (IC_{50} 2–3 μM), the growth of murine lymphocytic leukemia (L 1210 and P 388 with ED_{50} ~0.003 μM and human colon cancer cell lines [(e.g. LoVo (ED_{50} = 0.005 μg/ml), HT 29 (ED_{50} 0.02 μg/ml, Colo 205 (ED_{50} = 0.07 μg/ml), DLD-1 (ED_{50} = 0.005 μg/ml) and HCT-15 (ED_{50} = 0.0009 μg/ml)] designated combretastatin A-4 (**1c**). The structure assigned by spectral techniques was confirmed by synthesis.
Key words. Combretaceae; *Combretum caffrum*; combretastatin A-4; inhibitor tubulin; lymphocytic leukemia; human colon cancer.

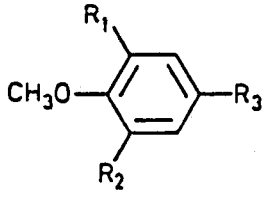
The willow-like appearance of the south African tree *Combretum caffrum* (shrub to 15 m high, Combretaceae) is a common sight overhanging stream beds, and the powdered root bark is used by the Zulu as a charm to harm an enemy [2]. Previously we reported the isolation of a series of bibenzyls [3], stilbenes [4, 5], and phenanthrenes [6] from this very productive tree. All were found active against the murine P 388 lymphocytic leukemia cell line, and some, especially combretastatins A-1 (**1a**) [4] and A-2 (**1b**) [5], were shown to be potent inhibitors of microtubule assembly. We now report that a trace fraction (26.4 mg from 77 kg of dry stem wood), selected due to inhibition of the P 388 cell line and tubulin polymerization, was found to contain a powerful inhibitor of microtubule assembly (IC_{50} 2–3 μM) named combretastatin A-4 (**1c**). The *cis*-stilbene (**1c**) was found comparable in its inhibitory effects to podophyllotoxin and combretastatin A-1 and more potent than colchicine and steganacin. An additional new compound, combretastatin A-5 (**1d**) was also isolated from this fraction, but it was significantly less active. The 0.65 g fraction that just preceded one employed to separate fractions containing combretastatins A-3 and B-2 [5] (during partition chromatography on Sephadex LH-20 in 3:1:1 hexane-toluene-methanol) was further refined by a series of liquid (3:1 and 4:1 hexane-ethyl acetate) chromatographic steps on silica gel and by HPLC (Partisil M-9, 9:1 hexane-2-propanol). While the resulting P 388 active fraction (26.4 mg) at first appeared homogeneous, high field (400 MH_z) ^{1}H-NMR suggested a mixture of at least three compounds that resisted separation until conversion (7.1 mg) to *t*-buthyldimethylsilyl ether derivatives (cf., **1e** and **1f**). Multiple development preparative thin layer chromatography on silica gel in 17:3 hexane-ethyl acetate afforded silyl ethers **1e** (3.0 mg, oil) and **1f** (2.0 mg, oil) corresponding, respectively, to combretastatins A-4 and A-5. The



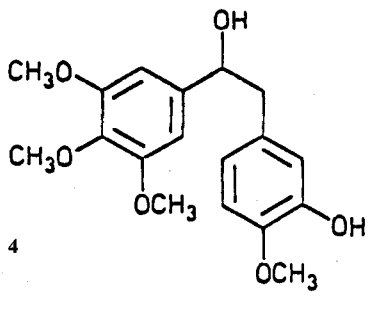
1a, $R_1 = R_2 = OCH_3$, $R_3 = H$, $R_4 = OH$
(Combretastatin A-1)
b, $R_1 = R_2 = OCH_2O$, $R_3 = R_4 = H$
(Combretastatin A-2)
c, $R_1 = R_2 = OCH_3$, $R_3 = R_4 = H$
(Combretastatin A-4)
d, $R_1 = OH$, $R_2 = OCH_3$, $R_3 = CH_3$, $R_4 = H$
(Combretastatin A-5)
e, $R_1 = R_2 = OCH_3$, $R_3 = Si(CH_3)_2C(CH_3)_3$, $R_4 = H$
f, $R_1 = OSi(CH_3)_2C(CH_3)_3$, $R_2 = OCH_3$, $R_3 = CH_3$, $R_4 = H$
g, $R_1 = R_2 = OCH_2O$, $R_3 = H$, $R_4 = OH$



2a, $R_1 = H$, $R_2 = CH_3$
(Combretastatin A-6)
b, $R_1 = Si(CH_3)_2C(CH_3)_3$, $R_2 = CH_3$
c, $R_1 = CH_3$, $R_2 = Si(CH_3)_2C(CH_3)_3$
d, $R_1 = CH_3$, $R_2 = H$



3a, $R_1 = OSi(CH_3)_2C(CH_3)_3$, $R_2 = H$, $R_3 = CH_2P^+(C_6H_5)_3Br^-$
b, $R_1 = R_2 = OCH_3$, $R_3 = CHO$
c, $R_1 = OSi(CH_3)_2C(CH_3)_3$, $R_2 = OCH_3$, $R_3 = CH_2P^+(C_6H_5)_3Br^-$
d, $R_1 = H$, $R_2 = OCH_3$, $R_3 = CHO$



4

third component designated combretastatin A-6 (**2a**), the *trans* isomer of A-5 (**1d**) was isolated as silyl ether **2b**, m.p. 116–117 °C (flakes from ethanol).

To confirm results of ^1H- and ^{13}C-NMR and mass spectral proposals for the structures of combretastatins A-4 to A-6, all were synthesized. Each synthetic *t*-butyldimethylsilyl ether derivative was compared with the natural counterpart and found to be identical. Synthesis of combretastatin A-4 was typical[4,5]. The ylide (n-butyl lithium) from phosphonium bromide **3a** (11.9 g) in tetrahydrofuran was treated (10 min) with aldehyde **3b** (3.1 g) to provide *cis* silyl ether **1e** (2.2 g, oil)[7] and *trans* isomer **2c** (2.98 g) as rods from ethanol, m.p. 128–130 °C following chromatographic (silica gel, 9:1 hexane-ethyl acetate) separation from the *Z*/*E* mixture (1.2 g, 93% overall yield) of isomers. Cleavage (tetrabutylammonium fluoride, 20 min, tetrahydrofuran) of silyl ether **1e** (1.7 g) followed by column chromatographic (silica gel, 3:2 hexane-ethyl acetate) purification yielded (1.15 g, 93%) combretastatin A-4: fine crystals from ethyl acetate-hexane; m.p. 84.5–85.5 °C; IR (NaCl) ν_{max} 3395, 1580, 1508, 1462, 1456, 1420, 1274, 1237, 1221, 1127, and 773 cm^{-1}.

An analogous route (1.5 g of **3c** + 0.33 g of **3d**) was employed to obtain 0.295 g of *Z*-isomer **1f** (oil) that upon cleavage of the silyl group provided (92%) combretastatin A-5 as an oil: IR (NaCl) ν_{max} 3424, 1601, 1583, 1512, 1463, 1429, 1268, 1259, 1237, 1140 and 1104 cm^{-1}. The *E*-isomers combretastatin A-6 (**2a**) and (**2d**) displayed respectively: granules from acetone-hexane, m.p. 122–124 °C; IR (NaCl) ν_{max} 3419, 1587, 1512, 1464, 1428, 1358, 1264, 1252, 1160, 1139 and 1104 cm^{-1} and amorphous solid m.p. 103–104 °C; IR (NaCl) ν_{max} 3422, 1585, 1509, 1463, 1454, 1418, 1333, 1277, 1261, 1253 and 1128 cm^{-1}. During NMR studies of combretastatin A-5 silyl ether (**1d**) in $CDCl_3$, its ready isomerization to *E*-isomer **2b** was observed.

Combretastatins A-4 to A-6 and *E*-isomers **2c** were all found to be significantly active against the U.S. National Cancer Institute's (NCI) murine L1210 and P388 lymphocytic leukemia cell lines. Most importantly, combretastatin A-4 was found to compete with the A-1[4] as the most potent inhibitor of colchicine binding to tubulin yet described, to be a stalwart inhibitor of tubulin polymerization (vide supra), to retard strongly (ED_{50} ~0.003 μM) of the murine lymphocytic leukemia L1210 and P388 cell lines as well as the LoVo [(ED_{50} = 0.005 μg/ml), HT29 (ED_{50} = 0.02 μg/ml), Colo 205 (ED_{50} = 0.07 μg/ml), DLD-1 (ED_{50} = 0.005 μg/ml) and HCT-15 (ED_{50} = 0.0009 μg/ml)] colon cancer cell lines, and to be the strongest antimitotic found among the *Combretum caffrum* constituents. For purposes of comparison, the heretofore unknown *cis*-stilbene analog **1g** (m.p. 124–126 °C as prisms from methylene chloride-hexane) of combretastatin A-1 was synthesized and found to inhibit tubulin polymerization with an IC_{50} value of 2–3 μM and the growth L1210 leukemia cells with an IC_{50} value of 4 μM, thus resembling combretastatins A-2 (**1b**, tubulin IC_{50} 4–5 μM, L1210 IC_{50} 0.1 μm) and A-1 (**1a**, tubulin IC_{50} 2–3 μM, L1210 IC_{50} 0.6 μM).

Biological evaluation (including in vivo) of combretastatin A-4 is continuing and will include the NCI astrocytoma bioassay where the close biosynthetic relative (−)-combretastatin (**4**)[2] was found to cause pronounced astrocyte reversal.

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1 For the preceding part (145) of the series Antineoplastic Agents refer to: H. Hennings, P. M. Blumberg, G. R. Pettit, C. L. Herald, R. Shores and S. H. Yuspa, *Carcinogenesis*, in press.

2 Pettit, G. R., Cragg, G. M., Herald, D. L., Schmidt, J. M., and Lohavanijaya, P., Can. J. Chem. *60* (1982) 1374.
3 Pettit, G. R., Singh, S. B., Niven, M. L., and Hamel, E., J. natl Prod. (1988) in press.
4 Pettit, G. R., Singh, S. B., Niven, M. L., Hamel, E., and Schmidt, J. M., J. natl Prod. *50* (1987) 119.
5 Pettit, G. R., and Singh, S. B., Can. J. Chem. *65* (1987) 2390.
6 Pettit, G. R., Singh, S. B., Niven, M. L., and Schmidt, J. M., Can. J. Chem. (1988) in press.
7 Predictable (ref. 4,5) ^{1}H-and ^{13}C-NMR, infrared and mass spectral data, as well as acceptable elemental analyses, were obtained for each new compound.

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Announcements

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