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# ANTIMALARIAL AGENTS, 4.[1] SYNTHESIS OF A BRUSATOL ANALOG AND BIOLOGICAL ACTIVITY OF BRUSATOL-RELATED COMPOUNDS

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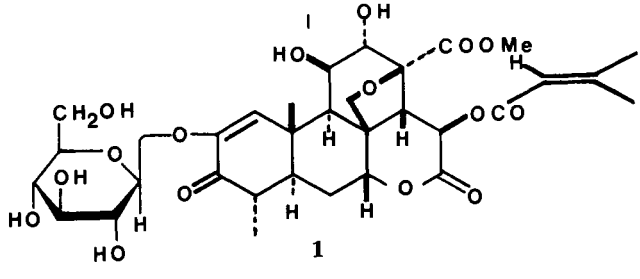
ABSTRACT.—The quassinoids bruceoside-A [**1**], brusatol [**2**], and bruceolide [**3**] were tested for antimalarial activity in vitro against the chloroquine-resistant (Smith) isolates of *Plasmodium falciparum*. Compound **2** was quite active, **1** was not active, and **3** showed only a trace of activity. The fact that 15 [(*E*)-non-2-enoyl] bruceolide [**7**] synthesized from **2** was eight times less active than **2** would indicate that the requirement of a C-15 ester moiety for enhanced antimalarial activity among brusatol related quassinoids could be quite specific.

The fruits of *Brucea javanica* (L.) Merr. (Simaroubacae), a plant occurring widely in South China, are known as "Ya Tan Tzu" in Chinese folklore and as herbal remedies for human cancer, amebiasis, and malaria (1-5). Notably, its water extract or powdered fruits are well known in China to be effective antimalarial drugs. According to many clinical reports (6-10), it has been concluded that the foregoing extract is highly active in human malaria, although it is toxic, including adverse reactions such as headache, nausea, vomiting, diarrhea, and weakness when used in high doses. Its antimalarial effect against chick malaria was reported to be comparable to quinine and superior to one of the oldest Chinese preparations, "Ch'ang Shan," which consists of the powdered roots of *Dichroa febrifuga* (11).

The potent antimalarial activity of *B. javanica* mentioned above, coupled with our recent isolation and characterization of its novel antileukemic principles (12-14), bruceoside-A [**1**] and -B, brucein-D and -E, cleomiscosin-A and brusatol [**2**], prompted our screening of **1**, **2**, and bruceolide [**3**], the hydrolyzed product of **2**, for potential antimalarial activity in vitro against the chloroquine-resistant (Smith) isolates of *Plasmodium falciparum*. A comparison of this preliminary assay demonstrated that **2** is quite active ($ED_{50}$=7.58 ng/ml), **1** is not active ($ED_{50}$=581.14 ng/ml), and **3** shows only a trace of activity ($ED_{50}$=75.20 ng/ml). This result clearly indicates the important contribution of the lipophilicity and/or other specific steric requirements, especially the C-15 ester moiety, to in vitro antimalarial activity. Thus, removal of this ester group of **2** reduced tenfold the activity as seen in **3**. Other earlier work on the antimalarial activity of bruceantin [**4**] whose C-15 ester side chain is slightly longer than **2** and bruceins-A [**5**] and -B [**6**], also supported this conclusion. Bruceantin is more than three times as

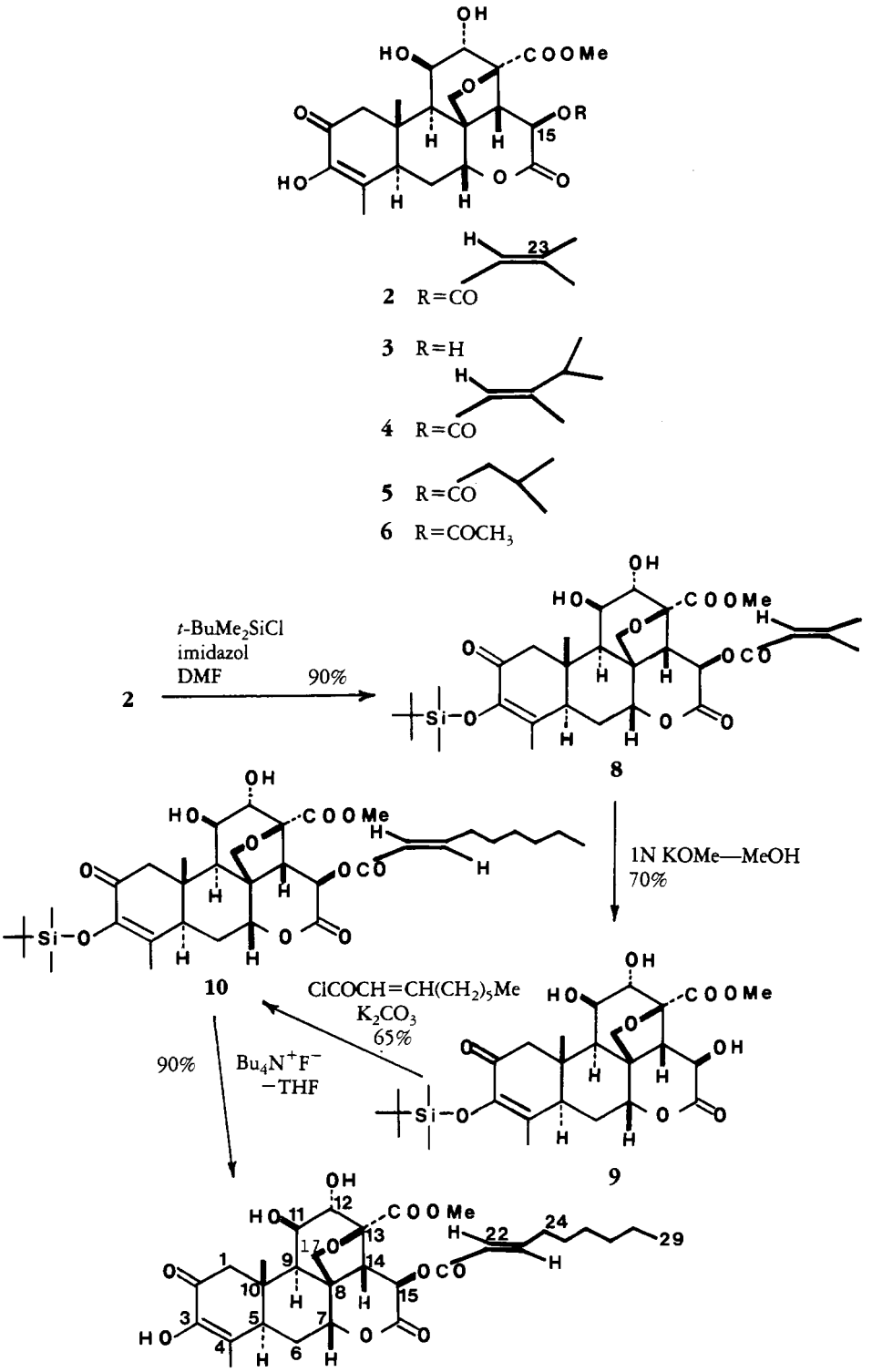



**1**

SCHEME 1

[1]For paper 3, see: Y. Zhao, I.H. Hall, C.B. Oswald, T. Yokoi, and K.H. Lee *Chem. Pharm. Bull.*, **35**, 2052 (1987).

SCHEME 1. *Continued.*



active as **2** (15) and is approximately ten times more active than **5** and **6** (16) in an in vitro assay against a chloroquine-resistant strain (K-1) of *P. falciparum*. Changes in the nature of this ester moiety obviously produce distinct alterations in the antimalarial activity. Thus, it appeared to be of interest to examine additional quassinoids with the introduction of different C-15 ester functionality as potential antimalarial agents. This paper describes the synthesis and the antimalarial activity of 15 [(*E*)-non-2-enoyl] bruceolide [**7**], derived from **2**.

## RESULTS AND DISCUSSION

CHEMISTRY.—Because bruceantin [**4**] is more active than brusatol [**2**], it would be of interest to study the effect of side-chain variation at C-24 upon the antimalarial activity. Thus, the target compound **7** was synthesized. The synthesis of **7** employed a procedure (Scheme 1) analogous to that reported by Honda *et al.* (17). Brusatol [**2**], obtained by hydrolysis of bruceoside-A [**1**] according to a literature method (13), was silylated by use of dimethyl-*t*-butylsilyl chloride to yield 89% of **8**. Alkaline hydrolysis of **8** with potassium methoxide gave rise to the alcohol **9** in 70% yield. Compound **9** was esterified with (*E*)-non-2-enoyl chloride to furnish **10** in 65% yield. Removal of the protecting group was achieved by treatment of **10** with tetrabutylammonium fluoride in tetrahydrofuran to afford **7** in 90% yield.

Compound **7** was evaluated for antimalarial activity[2] in vitro against the chloroquine-resistant (Smith) isolates of *P. falciparum*, using the semiautomated, microdilution technique according to Desjardins *et al.* (18), and was found to be eight times less active than **2**, as it showed an $ED_{50}$ value of 61.65 ng/ml, while **2** demonstrated an $ED_{50}$ value of 7.58 ng/ml in the same isolate. This result would indicate that the requirement of a lipophilic C-15 ester side chain for potential in vitro antimalarial activity among brusatol [**2**] related quassinoids could be quite specific. A correlation of the structure-activity relationships among **2-7** suggests that the introduction of rigid alkyl groups at C-23, such as the C-23-*gem*-dimethyl and C-23-*gem*-methyl and isopropyl groups as seen in **2** and **4**, respectively, contributes to enhanced antimalarial activity. Further investigation along this line is currently in progress.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer. $^1$H-nmr spectra were recorded on a Bruker 250 MHz spectrometer and are given in ppm (δ) downfield from an internal TMS standard. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Mass spectra were determined on a V.G. Micromass 70-70 instrument at 70 eV using a direct inlet system. Si gel for column chromatography refers to Merck Si gel 60 (70-230 mesh). Si gel for preparative tlc refers to Analtech Si gel G (1000 m). Compounds were visualized by uv light or spraying with 1% $Ce(SO_4)_2$-10%$H_2SO_4$ solution followed by heating. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

3-*O*-DIMETHYL-*t*-BUTYLSILYL BRUSATOL [**8**].—A mixture of brusatol [**2**] (1.04 g, 2 mmol) (13), dimethyl-*t*-butylsilyl chloride (360 mg, 2.4 mmol), and imidazole (340 mg, 5 mmol) in DMF (2 ml) was heated at 40-50° for 1.5 h. The reaction mixture was poured into $H_2O$, and the resulting crystals were filtered and recrystallized from $Et_2O$/petroleum ether to yield colorless crystals (1.14 g, 90% yield) of **8**: mp 242-243°; ir (KBr) 3500 (OH), 1735 (ester and lactone C=O), 1670 (α,β-unsat. C=O), 1610 (C=C) $cm^{-1}$; nmr ($CDCl_3$) δ 0.15 and 0.18 (3H each, s, $Me_2Si$), 0.96 (9H, s, $Me_3$ C-), 1.39 (3H, s, Me-10), 1.84 (3H, s, Me-4), 1.93 and 2.19 (3H each, s, $Me_2$-23), 2.32 and 2.93 (3H, ABq, $J$=15.2 Hz, $H_2$-1), 2.39 and 2.96 (1H each, d-like, $H_2$-6), 3.78 (3H, s, COOMe), 4.20 (1H, m, H-11), 4.26 (1H, m, H-12), 4.80 (1H, m, H-7), 5.62 (1H, s, H-22), 6.27 (1H, br. s, H-15). *Anal.* Found: C, 60.85; H, 7.26;

[2]The in vitro antimalarial assay was carried out by the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Institute of Research, Washington, DC.

calcd. for $C_{32}H_{46}O_{11}Si$: C, 60.50; H, 7.30%; ms *m/z* 619.2571 [calcd. for $C_{31}H_{43}O_{11}Si$ ($M^+$-Me): 619.2572].

3-*O*-DIMETHYL-*t*-BUTYLSILYL BRUCEOLIDE [**9**].—To a solution of **8** (500 mg, 0.78 mmol) in MeOH (30 ml) was added 1 N potassium methoxide in MeOH (100 ml) at 0°. After 12 h, the reaction mixture was neutralized by stirring with Dowex 50W-X2 cation exchange resin and evaporated in vacuo. The residue was extracted with $CHCl_3$, washed with $NaHCO_3$, $H_2O$, dried ($MgSO_4$), and distilled under reduced pressure to furnish **9** in 70% yield as amorphous powder: mp 158-160°; ir ($CHCl_3$) 3580 (OH), 1730 (ester and lactone C=O), 1670 (α,β-unsat. C=O), 1610 (C=C) $cm^{-1}$; nmr ($CDCl_3$) δ 0.13 and 0.18 (3H each, s, $Me_2Si$), 0.96 (9H, s, $Me_3C$-), 1.38 (3H, s, Me-10), 1.84 (3H, s, Me-4), 2.33 and 2.92 (2H, ABq, $J$=15.2 Hz, $H_2$-1), 2.37 and 2.90 (1H, each, d-like, $H_2$-6), 3.84 (3H, s, COOMe), 4.21 (1H, m, H-11), 4.26 (1H, m, H-12), 4.72 (1H, m, H-7), 5.28 (1H, d, $J$=11 Hz, H-15). *Anal.* Found: C, 58.90; H, 7.35; calcd. for $C_{27}H_{40}O_{10}Si$: C, 58.63; H, 7.30%; ms *m/z* 537.2157 [calcd. for $C_{26}H_{37}O_{10}Si$ ($M^+$-Me): 537.2153].

3-*O*-DIMETHYL-*t*-BUTYLSILYL-15[(*E*)-NON-2-ENOLYL]BRUCEOLIDE [**10**].—To 50 ml of a $CHCl_3$ solution of 15 [(*E*)-non-2-enoyl] chloride (900 mg, 5.1 mmol) (19), obtained from treatment of 2-nonenoic acid with thionyl chloride, was added dropwise **9** (720 mg, 1.3 mmol) in pyridine (2 ml). After the mixture was stirred for 1 h, it was refluxed for 5 h. The mixture was treated with dilute HCl to remove pyridine and then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with aqueous NaCl, $H_2O$, $NaHCO_3$, aqueous NaCl, and then $H_2O$, dried ($MgSO_4$) and evaporated in vacuo to yield a red oil. The product was separated from the reaction mixture by column chromatography over Si gel (10 g), eluted with $C_6H_6$ and then with $CHCl_3$-EtOAc (7:3). The eluate from the latter was dissolved in $Et_2O$, and then added to petroleum ether to yield a white powder (765 mg, 85% yield). Further purification of this product was achieved by preparative tlc [Si gel, $CHCl_3$-EtOAc (9:1), Rf=0.30] to furnish **10** as amorphous white powder (585 mg, 65% yield): mp 120-125°; ir ($CHCl_3$) 3700-3600 (OH), 1730 (C=O), 1670 (α,β-unsat. C=O), 1600 (C=C) $cm^{-1}$; nmr ($CDCl_3$) δ 0.15 and 0.16 (3H each, s, $Me_2Si$), 0.90 (3H, m, Me-29), 0.96 (9H, s, $Me_3C$-), 1.50-1.12 [8H, m, $(CH_2)_4$-25-28], 1.39 (3H, s, Me-10), 1.84 (3H, s, Me-4), 2.07 (1H, d, $J$=4.0 Hz, H-9), 2.22 (2H, q, $J$=7.5 Hz, $H_2$-24), 2.37 and 2.92 (2H, ABq, $J$=15.2 Hz, $H_2$-1), 2.41 and 2.96 (1H each, d-like, $H_2$-6), 3.13 (1H, d, $J$=15.0 Hz, H-14), 3.76 (3H, s, COOMe), 4.20 (1H, s, H-12), 4.26 (1H, br. d, $J$=4.0 Hz, H-11), 4.72 and 3.81 (1H each, d, $J$=7.5 Hz, H-17), 4.78 (1H, m, H-7), 5.77 (1H, d, $J$=17.0 Hz, H-22), 6.33 (1H, d, $J$=15.0 Hz, H-15), 7.05 (1H, sextet, $J$=17.0 and 7.5 Hz, H-23). *Anal.* Found: C, 62.80; H, 7.85; calcd. for $C_{36}H_{54}O_{11}Si$: C, 62.53; H, 7.88%, ms *m/z* 633.2750 (calcd. for $C_{36}H_{54}O_{11}Si$-57: 633.2728).

15[(*E*)-NON-2-ENOYL]BRUCEOLIDE [**7**].—To a solution of **10** (611 mg, 0.9 mmol) in anhydrous THF (1 ml) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride in THF (1.8 ml, 1.8 mmol). After the mixture was stirred for 15 min, it was poured into 30 ml of iced $H_2O$. The resulting oily substance was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with diluted HCl, $H_2O$, diluted $NaHCO_3$ and $H_2O$, dried ($MgSO_4$), and evaporated under reduced pressure to afford an oil (500 mg). This oil was dissolved in EtOAc (1 ml) and then added with petroleum ether to yield a precipitate. Further purification of this precipitate by washing with petroleum ether several times and drying gave rise to a colorless amorphous powder **7**, (460 mg, 90% yield): mp 110-115°; ir ($CHCl_3$) 3600-3400 (OH), 1750 (lactone C=O), 1730 (3ster C=O), 1640 and 1665 [CO-C(OH)=C], 1720 (C=C-COO) $cm^{-1}$; nmr ($CDCl_3$) δ 0.90 (3H, m, Me-29), 1.20-1.82 [8H, m, $(CH_2)_4$-25-28], 1.39 (3H, s, Me-10), 1.84 (3H, s, Me-4), 2.13 (1H, d, $J$=7.0 Hz, H-9), 2.22 (2H, q, $J$=7.5 Hz, $H_2$-24), 2.39 and 2.98 (1H, each, d-like, $H_2$-6), 2.43 and 2.99 (2H, ABq, $J$=15.2 Hz, $H_2$-1), 3.13 (1H, d, $J$=15.0 Hz, H-14), 3.36 (1H, br. s, OH-12), 3.76 (3H, s, COOMe), 4.20 (1H, s, H-12), 4.26 (1H, d, $J$=7.0 Hz, H-11), 3.81 and 4.72 (1H each, d, $J$=7.5 Hz, H-17), 4.78 (1H, m, H-7), 5.77 (1H, d, $J$=17.0 Hz, H-22), 6.09 (1H, br. s, OH-3), 6.33 (1H, d, $J$=15.0 Hz, H-15), 7.05 (1H, sextet, $J$=17.0 and 7.5 Hz, H-23). *Anal.* Found: C, 58.88; H, 6.86; calcd. for $C_{30}H_{40}O_{11}.2H_2O$: C, 58.81; H, 7.23%; ms *m/z* 576.2579 calcd. for $C_{30}H_{40}O_{11}$ ($M^+$): 576.2568].

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## LITERATURE CITED


1. "Ku Chin Chung Yao Chi Ch'eng," (Collection of Ancient and Modern Chinese Drugs), Ta Chung Shu Chu, 1957, p. 448.
2. W.S. Kan "Pharmaceutical Botany," National Research Institute of Chinese Medicine, Taiwan, Republic of China, 1969, p. 356.
3. M. Sugi and Y. Nagashio, in: "Cancer Therapy in Modern China." Ed. by J. Kondo, Shizen Sha, Japan, 1977, p. 109.
4. "Chung Yao Ta Tsu Dan" (Dictionary of Chinese Medicine), Vol. 2, Shanghai Science and Technology Publishing Co., 1978, p. 1642.
5. W.F. Fan, "I Yao Hsueh" (Medicine and Pharmacy), Vol. 5, and references cited therein, 1952, p. 28.
6. "I Wu Shen Huo," June, 1953, p. 10 (A report from the Malarial Therapeutic Hospital of Shanghai First Medical College at Fo-Tzu-Ling).
7. H. Chen, *J. Chinese Medicine*, **9**, 721 (1957).
8. "Chiang-Su I Hsueh Ko Chi Tzu Liao," (Chiang-Su Medical Science Information) **1**, 17 (1962).
9. "Chung Hua I Hsueh" (Chinese Medicine) **36**, 469 (1950).
10. "Ko Hsueh Tung Dao" (Scientific Communication), **1**, 460 (1950).
11. T.R. Sweeney and R.E. Strube, in: "Burger's Medicinal Chemistry," 4th ed., John Wiley & Sons, Vol. 1, Chapter 18, 1979, p. 333, and literature cited therein.
12. K.H. Lee, Y. Imakura, and H.C. Huang, *J. Chem. Soc., Chem. Commun.*, **1977**, 69.
13. K.H. Lee, Y. Imakura, Y. Sumida, R.Y. Wu, I.H. Hall, and H.C. Huang, *J. Org. Chem.*, **44**, 2180 (1979).
14. K.H. Lee, N. Hayashi, M. Okano, H. Nozaki, and M. Ju-ichi, *J. Nat. Prod.*, **47**, 550 (1984).
15. M.J. O'Neill, O.H. Bray, P. Boardman, J.D. Phillipson, D.C. Warhurst, W. Peters, and M. Suffness, *Antimicrob. Agents Chemother.*, **30**, 101 (1986), and literature cited therein.
16. M.J. O'Neill, P. Boardman, K.L. Chan, D.H. Bray, J.D. Phillipson, and D.C. Warhurst, *J. Pharm. Pharmac.*, **37**, suppl. 49 (1985).
17. T. Honda, K. Imao, M. Inoue, N. Nakazuka, T. Tachioka, and T. Nakanishi, *Abstracts of 1983 Annual Meeting of Japanese Pharmacy Society*, Tokyo, Japan.
18. R.E. Desjardins, C.J. Canfield, J.D. Haynes, and J.D. Chulay, *Antimicrob. Agents Chemother.*, **16**, 710 (1979).
19. P.A. Chaloner, V.J. Harding, and C. Weizmann, *J. Chem. Soc. Perkin Trans II*, **7**, 1028 (1980).


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