Antimalarial 5,6-Dihydro-α-pyrones from Cryptocarya rigidifolia: Related Bicyclic Tetrahydro-α-Pyrones Are Artifacts

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Supporting Information

ABSTRACT: Antimalarial bioassay-guided fractionation of an EtOH extract of the root wood of Cryptocarya rigidifolia (Lauraceae) led to the isolation of the five new 5,6-dihydro-α-pyrones cryptorigidifoliols A–E (1–5) and the six bicyclic tetrahydro-α-pyrone derivatives cryptorigidifoliols F–K (6–11). The structure elucidations of all compounds were made on the basis of the interpretation of spectroscopic data and chemical derivatization, and the relative and absolute configurations were determined by NOESY, electronic circular dichroism (ECD), and 1H NMR analysis of α-methoxyphenylacetyl (MPA) derivatives. The bicyclic tetrahydro-α-pyrene derivatives were identified as products of acid-catalyzed intramolecular Michael addition of the 5,6-dihydro-α-pyrones in the presence of silica gel. A structure–activity relationship study suggested that the presence of an αβ-unsaturated carbonyl moiety is not essential for potent antimalarial activity.

As a part of the Madagascar International Cooperative Biodiversity Group (ICBG) program, an EtOH extract of the root wood of Cryptocarya rigidifolia (Lauraceae) was selected for bioassay-directed fractionation because of its reproducible activity against Plasmodium falciparum Dd2 (IC50 ~5 μg/mL). The genus Cryptocarya is distributed throughout the tropic, subtropic, and temperate regions of the world, and its members produce an array of secondary metabolites including flavonoids such as cryptochinones,1 which have recently been shown to act as farnesoid X receptor agonists,4 alkaloids,5 and a variety of 5,6-dihydro-α-pyrones6–11 some of which have the ability to stabilize the tumor suppressor PDCD4.12 Other Cryptocarya-derived α-pyrones display antiparasitic,13 antimycobacterial,15 antitumor,14 and anticancer activities.15–17

A combination of liquid–liquid partition, open-column chromatography, solid-phase extraction (SPE), HPLC, and preparative TLC afforded a series of new 5,6-dihydro-α-pyrones (1–5) and bicyclic tetrahydro-α-pyrene derivatives (6–11) from the root wood of C. rigidifolia. As explained below, compounds 6–11 were not found in the crude EtOH extract but were shown to be produced by cyclization of 5,6-dihydro-α-pyrones during the isolation process. 5,6-Dihydro-α-pyrones have been isolated from several members of the genus Cryptocarya, whereas the bicyclic pyrones have only been isolated from C. latifolia,8 C. myrtifolia,7 Polyalthia parviflora, and the Chinese medicinal ants Polyrhacis lamellidens.18 All of the reported isolation and purification procedures that yielded the bicyclic pyrones involved chromatography on silica gel at some stage, and our studies suggest that these bicyclic pyrones may also be artifacts.7,8,18,19

RESULTS AND DISCUSSION

The EtOAc-soluble fraction obtained from a liquid–liquid partition of the EtOH extract (100 mg) showed antiplasmodial activity. Dereplication as previously described20 indicated that the extract contained at least one new bioactive compound, so a larger sample was investigated. Fractionation of the EtOAc-soluble fraction of this sample by chromatography on Sephadex LH-20, reverse-phase SPE, normal-phase silic-gel column chromatography, and C18 HPLC yielded compounds 1 and 6–8, together with fractions that were mixtures of 5,6-dihydro-α-pyrones and bicyclic tetrahydro-α-pyrones. Purification of these fractions was effected by diol PTLC or HPLC to yield

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Compounds 2, 3, 5, and 9–11. Similar fractionation of the antiplasmodial hexanes fraction yielded compounds 4 and 7.

![Figure 1. Key HMBC correlations of 1.](image)

These data indicated a six-substituted 5,6-dihydro-α-pyrone, a common ring system of secondary metabolites found in Cryptocarya species. The remaining oxymethylene group at δH 5.34 (m, 1H, H1′) was assigned to a hydroxy group and an α,β-unsaturated δ-lactone moiety, respectively. Its 1H NMR spectroscopic data had signals for a conjugated olefinic moiety at δH 6.90 (1H, H4) and 6.03 (dt, J = 9.8, 1.7 Hz, 1H, H3), two oxymethylene groups at δH 4.75, 4.00 (1H, H2′ and 1H, H2′′) in the upfield region, and a multiplet at δH 2.36 (2H, H5), representing a de-shielded methylene group. The HMBC spectrum, the methylene protons at δH 2.36 (H5) showed correlations both to an olefin group (δC 121.3, C3, and 145.0, C4) and to the oxymethine resonance at δH 4.75 and δC 74.8 (C6). In addition, both the olefinic protons (δH 6.90, H4, and δH 6.03, H3) and the oxymethylene at δH 4.75 (H6) correlated with a carbonyl carbon at δC 164.6 (C2) (Figure 1).

The molecular formula of compound 3 (C22H38O3, HRESIMS m/z 351.2902 [M + H]+) and its 1H NMR spectrum (δH 5.34, 5.68, 6.03, 6.90, 7.00, and 9.99, 1H, H1′, H10′ and H11′) indicated that it had an additional disubstituted olefinic moiety as compared with compound 1. Its UV, IR, and 1H NMR spectroscopic data indicated the presence of the same α-pyrone ring as in 1 and 2, and HMBC correlations from H10′ and H11′ (δH 5.34) to the carbons at δC 7.0 indicated that the additional olefinic moiety must be located between two methylene groups. Long-range correlation from both H9′ and H12′ (δH 2.05–1.99, m, 4H) and H8′ and H13′ (δH 1.37–1.31, 1H, m, 4H) to the carbons at C10′ and C1′ (δC 130) in the HMBC spectrum assigned the olefinic moiety to C10′ and C1′. The geometry of the double bond was assigned as Z on the basis of the shielded 13C NMR chemical shift of the methylenes connected to the double bond (δC 29.4).14,26 The position of the double bond within the alkyl chain was determined unambiguously by analysis of the GC-EIMS fragmentation of the dimethyldisulfide (DMDS) derivative of 327,28 which showed a major ion at m/z 299 attributable to fragmentation between the two CHS groups located at the original site of unsaturation. Fragment ions at m/z 281 and 145 were also observed and support the assigned structure (Figure S2a). The relative configuration of 3 and the assignment of its 1H and 13C NMR data were determined by the same methods as for 1 and 2. Compound 3 was assigned as 6R-(2′R-α-hydroxy-10′Z-heptadecenyl)-5,6-dihydro-2H-pyran-2-one22,25 and is named cryptotrigidifol D.

The molecular formula of 4 (C24H42O4, HRESIMS m/z 396.3489 [M + NH4]+) differed from the molecular formula of 3 (C22H38O3) only by a C4H4 unit. Inspection of the 1H NMR spectra of the DMDS adduct of 4 indicated that the position of the double bond was between C10′ and C11′ (Figure S2b). The complete NMR data and configurations of all stereogenic centers in 4 were assigned by the same methods as for 1–3. Compound 4 was assigned as 6R-(2′S-hydroxy-10′Z-nonadecenyl)-5,6-dihydro-2H-pyran-2-one22,28 and is named cryptotrigidifol C.

The 1H NMR spectrum of compound 5 (C28H44O4, HRESIMS m/z 377.3060 [M + OH]+) showed the presence of two oxymethylene groups (δH 4.63, 6.1H, H2′; δH 4.15, 1H, H12′) and a double bond (δH 5.68, 1H, H4′; δH 5.49, dd, J = 15.3, 7.0, 1H, H3′) in the alkyl chain, besides the α-pyrene signals at δH 6.90, 6.03, 4.69, and 2.44. The large coupling constant (15.3 Hz) observed for H3′ indicated the E geometry of the double bond. In the HMBC spectrum,
Table 1. $^1$H and $^{13}$C NMR Spectroscopic Data for Compounds 1–5$^a$

| posn | $\delta^b$ | $\delta^c$ | posn | $\delta^b$ | $\delta^c$ | posn | $\delta^b$ | $\delta^c$ | posn | $\delta^b$ | $\delta^c$ | posn | $\delta^b$ | $\delta^c$ |
|------|-----------|-----------|------|-----------|-----------|------|-----------|-----------|------|-----------|-----------|------|-----------|-----------|
| 2    | 164.6 (C) | 164.5 (C) | 12   | 1642 (CH$_2$) | 150.3 (CH$_2$) | 21   | 163.5 (C) | 150.3 (CH$_2$) | 26   | 164.6 (C) | 164.5 (C) |
| 3    | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 17   | 6.04 dt (9.8, 1.7) | 121.3 (CH) | 27   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 21   | 1642 (CH$_2$) | 150.3 (CH$_2$) |
| 4    | 6.90 m | 145.0 (CH) | 18   | 6.90 m | 145.0 (CH) | 22   | 6.90 m | 145.0 (CH) | 22   | 2.04 m | 39.6 (CH$_2$) |
| 5    | 2.36 m | 30.1 (CH$_3$) | 19   | 2.36 m | 30.1 (CH$_3$) | 23   | 2.36 m | 30.1 (CH$_3$) | 23   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) |
| 6    | 4.75 m | 74.8 (CH) | 20   | 4.75 m | 74.8 (CH) | 24   | 4.75 m | 74.8 (CH) | 24   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) |
| 7    | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) | 21   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) | 25   | 1.96 ddd (14.5, 9.6, 22) | 42.1 (CH$_3$) | 25   | 1.96 ddd (14.5, 9.6, 22) | 42.1 (CH$_3$) |
| 8    | 1.96 ddd (14.5, 9.6, 22) | 42.1 (CH$_3$) | 26   | 1.96 ddd (14.5, 9.6, 22) | 42.1 (CH$_3$) | 26   | 1.96 ddd (14.5, 9.6, 22) | 42.1 (CH$_3$) |
| 9    | 2.04 m | 39.6 (CH$_2$) | 27   | 2.04 m | 39.6 (CH$_2$) | 27   | 2.04 m | 39.6 (CH$_2$) | 27   | 2.04 m | 39.6 (CH$_2$) |
| 10   | 1.79 ddd (14.3, 5.6, 39) | 1.64 ddd (14.5, 102, 29) | 28   | 6.91 m | 181 (CH$_2$) | 28   | 6.91 m | 181 (CH$_2$) | 28   | 6.91 m | 181 (CH$_2$) |
| 11   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 29   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 29   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 29   | 6.03 dt (9.8, 1.7) | 121.3 (CH) |
| 12   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 30   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 30   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 30   | 6.03 dt (9.8, 1.7) | 121.3 (CH) |
| 13   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 31   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 31   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 31   | 6.03 dt (9.8, 1.7) | 121.3 (CH) |
| 14   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 32   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 32   | 6.03 dt (9.8, 1.7) | 121.3 (CH) | 32   | 6.03 dt (9.8, 1.7) | 121.3 (CH) |
| 15   | 2.04 m | 39.6 (CH$_2$) | 33   | 2.04 m | 39.6 (CH$_2$) | 33   | 2.04 m | 39.6 (CH$_2$) | 33   | 2.04 m | 39.6 (CH$_2$) |
| 16   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) | 34   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) | 34   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) |
| 17   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) | 35   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) | 35   | 1.92 ddd (14.5, 9.6, 22) | 41.2 (CH$_2$) |
| 18   | 2.04 m | 39.6 (CH$_2$) | 36   | 2.04 m | 39.6 (CH$_2$) | 36   | 2.04 m | 39.6 (CH$_2$) | 36   | 2.04 m | 39.6 (CH$_2$) |
| 19   | 1.64 ddd (14.5, 102, 29) | 1.64 ddd (14.5, 102, 29) | 37   | 1.64 ddd (14.5, 102, 29) | 1.64 ddd (14.5, 102, 29) | 37   | 1.64 ddd (14.5, 102, 29) | 1.64 ddd (14.5, 102, 29) |

$^a$Spectra obtained in CDCl$_3$; assignments on the basis of analysis of 2D NMR spectra. $^b$Data (δ) measured at 500 MHz; brs = broad singlet, brd = broad doublet, t = triplet, ddd = doublet of doublets of doublets, dt = doublet of triplets, m = multiplet. $^c$Values are in Hz and are omitted if the signals overlapped as multiplets. The overlapped signals were assigned from HSQC and HMBC spectra without designating multiplicity. $^d$Data (δ) measured at 125 MHz; CH$_3$, CH$_2$, CH, and C multiplicities were determined by an HSQC experiment.
correlations were observed between the protons at δ_H 1.79 and 1.73 (each m, 1H, H1’) and C3’ (δ_c 131.4) and between the proton at δ_H 4.63 (m, 1H, H2’) and C4’ (δ_c 132.6). These observations suggested the connection of the olefinic moieties with the C2’ oxime thione functionality. The EIMS data of S showed significant ions at m/z 265 and 247, together with a less intense ion at m/z 111, consistent with assignment of the second hydroyx group to C12’ (Figure S3). The configurations at C6 and C2’ were assigned to be R and S, respectively, by interpretation of the ECD spectroscopic data and by the MPA ester method. An attempt was made to determine the configuration at C12’ by using the MPA ester method, but it did not lead to any firm conclusion because we could not distinguish the chemical shifts of the protons of the two methylene groups attached to C12’. Compound S was assigned as 6R-(2’R,12’C)-di hydroyx-3’E-nonadecenyl)-5,6-di hydroy-2H-pyran-2-one and named cryptorigidifoliol E.

Compound 6 had the molecular formula of C_{24}H_{42}O_{4} on the basis of its HRESIMS (m/z 393.3419 [M + H]^+). Its IR spectrum showed the absorptions characteristic of a δ-lactone moiety (1719 and 1073 cm^{-1}). The 1H NMR spectrum lacked the signals for the vinylic protons of an αβ-unsaturated lactone unit, and the IR absorption at 1615 cm^{-1} found in compounds 1–S was absent. A new methylene signal was observed at δ_H 2.89 (brd, J = 19.3 Hz, 1H, H4a) and 2.78 (dd, J = 19.3, 4.5 Hz, 1H, H4b), which showed HMBC correlations with a carbonyl carbon at δ_c 169.7 (C3, Figure 2). A signal for a vinylic proton was observed in the 1H NMR spectrum at δ_H 5.33 (m, 2H, H8’ and H9’), and the four indices of hydrogen deficiency of 6 thus required a second ring in addition to the lactone and the double bond functionalities. The 1H NMR spectrum showed the presence of four oxime thione protons (δ_H 4.89 (m, 1H, H1), 4.36 (t, J = 4.5 Hz, 1H, H5), 4.10 (m, 1H, H7) and 3.81 (m, 1H, H2’)) directly attached to C1 (δ_c 73.1), C5 (δ_c 66.0), C6 (δ_c 63.6), and C2’ (δ_c 68.3), respectively, assignments that were confirmed by HSQC data. In the HMBC spectrum, cross peaks were observed from the two oxime thione protons at δ_H 4.89 (H1) and 4.36 (H5) to the carbonyl carbon at δ_c 169.7 (C3) and the C7 oxime thione (δ_c 63.6). Correlations were also observed between the two oxime thione protons H1 and H5 and C5 (δ_c 66.0) and C1 (δ_c 73.1), respectively, and between the oxime thione proton at δ_H 4.10 (H7) and the oxygenated carbon (δ_c 68.3 (C2’); δ_c 3.81 (m, 1H, H2’); Figure 2). Collectively, these correlations permitted assignment of the bicyclic ring system, the alkyl chain substituted at C7, and a hydroxy group at C2’ of 6 (Figure 2).

The long-range cross peaks observed between H8’ and H9’ (δ_H 5.33, m, 2H) and δ_c 29.6 in the HMBC spectrum indicated that the allylic methylene groups resonated at δ_c 29.6 (C7’ and C10’) and indicated the Z geometry of the sole double bond in the alkyl chain. The position of this double bond was assigned to C8’9’ on the basis of the EIMS fragmentation of the DMDS adduct, which had an intense peak at m/z 173 and a peak at m/z 297 corresponding to loss of water from the lactone-containing fragment ion (Figure S2c).

An attempt to assign the configuration at C2’ of 6 by the MPA ester method was not successful because there were no significant Δδ (H) differences between its R- and S-MPA derivatives. Because bicyclic tetrahydroy-α-pyrones such as 6 are formed from the corresponding 5,6-dihydroy-α-pyrones, as explained below, and all of these 5,6-dihydroy-α-pyrones have the 6R configuration, it is proposed that the orientation of substituents at C1 of 6 (corresponding to C6 in the putative monocyclic precursor) must be the same. Because of a change in group priorities, the C1 absolute configuration is S. The preference for the formation of a less-strained cis fused bicyclic system dictates the generation of a 5R-configured center. The same cis fused configuration was demonstrated for some related bicyclic tetrahydroy-α-pyrones by X-ray crystallography.\(^{10,29}\) The configuration of 6 at C7 could not be assigned because its 5,6-dihydroy-α-pyrone precursor was not isolated. The complete NMR assignments of 6 (Table 2) were facilitated by further interpretation of its HMBC and HSQC spectra. Compound 6 was thus assigned as (1S,5R,7’R)-7’-(2’-hydroxy-8’-Z-heptadecenyl)-2,6-dioxacyclo[3.3.1]nonan-3-one and has been named cryptorigidifoliol F.

Compounds 7 (m/z 421.3662 [M + H]^+) and 8 (m/z 355.2831 [M + H]^+) also contained a bicyclic tetrahydroy-α-pyrene ring. Comparison of their molecular weights and 1H NMR spectra with those of cryptorigidifoliol F (6) revealed that the only structural differences were in the alkyl chain length and the absence or presence of a double bond or hydroxy group on the alkyl chain. Cryptorigidifoliol G (7), with the molecular formula C_{24}H_{42}O_{7}y, was a bicyclic tetrahydroy-α-pyrene similar to 6 but lacked the C2’ hydroy group and had a 20-carbon alkyl chain at C7. Preparation of its DMDS derivative permitted assignment of the double bond at C8’ (m/z at 299 and 215; Figure S2c). Its configuration at C7 could not be determined because its presumed monocyclic precursor was not isolated. Cryptorigidifoliol H (8) was also a bicyclic tetrahydroy-α-pyrene similar to 6 but with a saturated alkyl chain at C7; the chain length was determined to be 14C on the basis of its molecular formula of C_{24}H_{42}O_{6}. As in the case of 7, its configuration at C7 could not be determined.

Compounds 9–11 (cryptorigidifoliols I–K) are the bicyclic derivatives of 2, 3, and S, as determined by the conversions described below and by comparison of their 1H NMR and HRESIMS data with those of their 5,6-dihydroy-α-pyrene precursors and those of compounds 6–8. Because the configurations of the precursors 2, 3, and S at the 2’ position have been established, the corresponding configurations at C7 of 9, 10, and 11 were assigned as S, R, and R, respectively. Compounds 6, 8, 9, and 11 had no significant Δδ (H) difference between their R- and S-MPA derivatives, thus precluding assignment of absolute configurations at these centers.

**Evidence that Bicyclic Tetrahydropyrones 6–11 Are Artifacts.** The initial purification of the bioactive EtOAc fraction involved Sephadex LH-20 column chromatography, reverse-phase SPE, normal-phase silica-gel column chromatography, and C_{18} HPLC. This procedure yielded fractions that in most cases were mixtures of 5,6-dihydro-α-pyrones and bicyclic tetrahydropyrones. In addition, it was noted that the early-eluting fractions from the open silica-gel column contained either pure 5,6-dihydroy-α-pyrene 1 or mixtures of 5,6-dihydroy-α-pyrones 2, 3, and S and bicyclic tetrahydropyrones 9–11. The later-eluting fractions, however, yielded only the bicyclic tetrahydropyrones 6–8. This suggested that the 5,6-dihydroy-α-pyrones were cyclized during their exposure to silicone gel,
Table 2. $^1$H and $^{13}$C NMR Spectroscopic Data for Compounds 6–8 and $^1$H NMR Spectroscopic Data for Compounds 9–11 $^a$

| posn | $\delta_H^b$  | $\delta_C^c$  | $\delta_H^b$  | $\delta_C^c$  | $\delta_H^b$  | $\delta_C^c$  | $\delta_H^b$  | $\delta_C^c$  | $\delta_H^b$  | $\delta_C^c$  | $\delta_H^b$  | $\delta_C^c$  |
|-------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1     | 4.89 m         | 73.1 (CH)      | 4.89 m         | 73.1 (CH)      | 4.89 m         | 73.1 (CH)      | 4.89 m         | 73.1 (CH)      | 4.89 m         | 490 m          | 4.89 m         | 490 m          |
| 3     | 1.41 m         | 29.4 (CH₂)     | 1.51 m         | 29.4 (CH₂)     | 1.41 m         | 29.4 (CH₂)     | 1.38 m         | 1.51 m         | 1.30 m         | 1.30 m         | 1.30 m         | 1.30 m         |
| 4     | 4.36 t (4.5)   | 66.0 (CH)      | 4.35 brs       | 66.0 (CH)      | 4.36 t (4.5)   | 66.0 (CH)      | 4.41 brs       | 4.34 brs       | 4.40 brs       | 4.40 brs       | 4.40 brs       | 4.40 brs       |
| 5     | 4.10 m         | 63.6 (CH)      | 4.02 m         | 3.73 brs       | 4.27 m         | 63.6 (CH)      | 4.02 m         | 3.73 brs       | 4.27 m         | 1.92 m         | 37.8 (CH₂)     | 1.95 m         |
| 6     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    |
| 7     | 1.38–1.28 m    | 27.3 (CH₃)     | 1.35–1.22 m    | 27.3 (CH₃)     | 1.35–1.22 m    | 27.3 (CH₃)     | 1.35–1.22 m    | 27.3 (CH₃)     | 1.35–1.22 m    | 27.3 (CH₃)     | 1.35–1.22 m    |
| 8     | 1.46–1.38 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    |
| 9     | 2.07–1.97 m    | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     | 1.35–1.22 m    | 29.6 (CH₂)     |
| 10    | 1.29–1.19 m    | 14.2 (CH₃)     | 1.29–1.19 m    | 14.2 (CH₃)     | 1.29–1.19 m    | 14.2 (CH₃)     | 1.29–1.19 m    | 14.2 (CH₃)     | 1.29–1.19 m    | 14.2 (CH₃)     | 1.29–1.19 m    |
| 11    | 0.88 t (7.0)   | 14.2 (CH₃)     | 0.88 t (7.0)   | 14.2 (CH₃)     | 0.88 t (7.0)   | 14.2 (CH₃)     | 0.88 t (7.0)   | 14.2 (CH₃)     | 0.88 t (7.0)   | 14.2 (CH₃)     | 0.88 t (7.0)   |

$^a$Spectra obtained in CDCl₃; assignments are on the basis of analysis of 2D NMR spectra. $^b$Data ($\delta$) measured at 500 MHz; s = singlet, br d = broad doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplet. J values are in Hz and are omitted if the signals overlapped as multiplets. The overlapped signals were assigned from HSQC and HMBC spectra without designating multiplicity. $^c$Data ($\delta$) measured at 125 MHz; CH₃, CH₂, CH, and C multiplicities were determined by an HSQC experiment.
the more polar later-eluting compounds that were exposed for a longer time to silica gel becoming completely converted to cyclized product. Pure 5,6-dihydro-α-pyrones 1 and related compounds were thus prepared by purification by PTLC on diol silica gel, which did not induce intramolecular cyclization. It is worth noting that Girkovic and co-workers isolated only 5,6-dihydro-α-pyrones, without artifacts, by using diol column chromatography during their bioassay-guided fractionation of a Papua New Guinea species of Cryptocarya in order to find compounds that can rescue Pdc4 from TPA-induced degradation.14

To verify the cyclization hypothesis, silica gel was mixed with compound 2 in MeOH/hexanes/EtOAc and the resulting suspension was allowed to stand for 3 h at room temperature. Examination of the resulting solution showed that only compound 9 was present, confirming that silica gel catalyzed the cyclization of the 5,6-dihydro-α-pyrene 2 to the bicyclic tetrahydropyrene 9 (Scheme 1). Compounds 3 and 5 were also treated in the same way, leading to the formation of the corresponding bicyclic tetrahydropyrene derivatives 10 and 11. These observations supported the hypothesis that the bicyclic tetrahydropyrones are formed by intramolecular cyclization of C2′-hydroxylated 5,6-dihydro-α-pyrones. However, this does not rule out the possibility that at least some bicyclic tetrahydropyrones are formed in the plant, and examination of the crude extract by 1H NMR spectroscopy indicated that it did contain signals consistent with the presence of bicyclic tetrahydropyrones. This result could be due to either the presence of the cyclized compounds in the plant or intramolecular cyclization during the extraction of the plant and the processing of the extract in Madagascar prior to analysis. Regrettably, we did not have access to fresh plant material to test these alternate hypotheses, but the fact that chromatography over silica gel was used in every case where the cyclized compounds are reported strongly suggests that these compounds are indeed formed during the purification process.

**Biological Activities.** Compounds 1–11 were evaluated for their antiparasitic activity against the chloroquine/melquine-resistant Dd2 strain of *Plasmodium falciparum*.

| Table 3. Bioactivities of 5,6-Dihydro-α-pyrones 1–5 |
|-----------------------------------------------------|
| **Compound** | **Dd2 IC_{50} (µM)\textsuperscript{a}** | **A2780 IC_{50} (µM)\textsuperscript{b}** |
| --------------|-----------------|-----------------|
| 1             | 9.2 ± 0.9       | >10             |
| 2             | 5.8 ± 1.4       | >10             |
| 3             | 5.5 ± 0.7       | 8.6             |
| 4             | 7.4 ± 0.6       | >10             |
| 5             | 9.0 ± 3.0       | >10             |
| artemisinin    | 0.007 ± 0.001   | N/A             |
| taxol         | 0.028 ± 0.002   |                 |

\(\textsuperscript{a}\) Antimalarial activity against the Dd2 strain of *Plasmodium falciparum*  
\(\textsuperscript{b}\) Antiproliferative activity against human ovarian cancer cells.

| Table 4. Bioactivities of Bicyclic Tetrahydro-α-pyrene Derivatives 6–11 |
|---------------------------------------------------------------|
| **Compound** | **Dd2 IC_{50} (µM)\textsuperscript{a}** | **A2780 IC_{50} (µM)\textsuperscript{b}** |
| --------------|-----------------|-----------------|
| 6             | 4.0 ± 2.0       | >10             |
| 7             | 6.0 ± 0.5       | >10             |
| 8             | >10             | >10             |
| 9             | >10             | >10             |
| 10            | >10             | >10             |
| 11            | >10             | >10             |
| artemisinin    | 0.007 ± 0.001   | N/A             |
| taxol         | 0.028 ± 0.002   |                 |

\(\textsuperscript{a}\) Antimalarial activity against the Dd2 strain of *Plasmodium falciparum*  
\(\textsuperscript{b}\) Antiproliferative activity against human ovarian cancer cells.

Compounds 1–7 exhibited moderate antimalarial activity, with IC_{50} values of 9.2 ± 0.9, 5.8 ± 1.4, 5.5 ± 0.7, 9.0 ± 3.0, 4.0 ± 2, 7.4 ± 0.6, and 6.0 ± 0.5 µM, respectively. Compounds 8–11 were all less active, with IC_{50} values >10 µM (Tables 3 and 4). These data indicate that the presence of the α,β-unsaturated carbonyl moiety is not essential for antimalarial activity because bicyclic compounds 6 and 7 have activity comparable to that of α,β-unsaturated carbonyl compounds 1–5.

All of the compounds were also evaluated for their antiproliferative activity against A2780 human ovarian cancer cells, but only compound 3 had an IC_{50} value less than 10 µM (Tables 3 and 4). Because these compounds have only moderate antiparasitic activities, significant improvement in their potency and therapeutic index will be necessary before they can be used as lead compounds for the development of new antimalarial drugs.

The formation of compounds 6–11 serves to highlight the fact that 5,6-dihydro-α-pyrones containing C2′ hydroxy groups in the side chain are susceptible to cyclization in the presence of silica gel at room temperature. It is thus possible that the bicyclic tetrahydropyrones previously reported\(\textsuperscript{7,8,18,30}\) were also formed by cyclization of the corresponding 5,6-dihydro-α-pyrones during the isolation process. This finding also provides support for the general belief that silica gel should be avoided for the isolation of most natural products because it will not in general be known whether or not the unknown compounds might be susceptible to similar unwanted reactions. In place of silica gel, diol and C18 media are suitable for acid-sensitive compounds such as 5,6-dihydro-α-pyrones.

### EXPERIMENTAL SECTION

**General Experimental Procedures.** IR and UV spectra were measured on an MDIAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. 1D and 2D NMR spectra were recorded on a Bruker Avance 500 spectrometer in CDCl3 or pyridine-d5 (with CDCl3, or pyridine-d5, as reference). High-resolution ESI mass spectra were obtained on an Agilent 6220 mass spectrometer. ECD spectra were obtained on a JASCO J-815 instrument. Optical rotations were recorded on a JASCO P-2000 polarimeter. Open-column
chromatography was performed using Sephadex LH-20 and silicone gel (230-400 mesh, Silicycle Co., USA). Semi-preparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a semi-preparative Phenomenex C18 column (5 µm, 250 × 10 mm²) and semi-preparative Varian diol column (250 × 10.0 mm²), a Shimadzu SPD M10A diode array detector, and a SCL-10A system controller. Preparative HPLC was performed using Shimadzu LC-8A pumps coupled with a preparative Varian Phenomenex C18 column (250 × 21.4 mm²), a Shimadzu SPD M10A diode array detector, and an SCL-10A system controller. Preparative TLC was performed using diol plates (~500 m²/g, SorbTec Co., USA). All isolated compounds were purified to 95% purity or better, as judged by HPLC (both UV and ELSD detection) before determining bioactivity.

**Plant Material.** Plant parts of *Cryptocarya rigidifolia* van der Werff (Lauraceae) were collected and authenticated by F.R. and co-workers at an elevation of about 1000 m from a 13 m tall tree with a diameter of 13 cm at chest height. The tree had round green fruit, with the remains of brown calyx above. Collection was made near the towns of Imerimandros and Antanandava in the district of Ambatondrazaka, north of the Zahamena National Park, at coordinates 17°28’45” S, 48°44’10” E. The tree was growing in medium-altitude rainforest on the edge of an old area of slash-and-burn agriculture. Duplicate voucher specimens (Ratovoson 250) were deposited at the Centre National d’Application des Recherches Pharmaceutiques (CNARP), the Herbarium of the Department of Forestry and Fishery Research (TEFIR), and the Missouri Botanical Garden, St. Louis, Missouri (MO).

**Extraction and Isolation.** A ground sample of *C. rigidifolia* root wood (310 g) was extracted with EtOH (1000 mL) at room temperature to yield 16.3 g of crude EtOH extract designated MG 0441, and a portion of this extract was made available to Virginia Tech for bioassay-guided isolation. The crude EtOH extract (5.0 g) was dissolved in 90% aqueous MeOH (60 mL) and extracted with hexanes. The 90% aqueous MeOH layer was evaporated, suspended in H₂O (100 mL), and extracted with EtOAc (100 mL). The antimarial activities were concentrated in the EtOAc (IC₅₀ = 2.5–5 µg/mL) and hexanes fractions (IC₅₀ > 5 µg/mL). The EtOAc fraction (971 mg) was subjected to Sephadex LH-20 size chromatography (CH₂Cl₂/MeOH = 1:1) to give four fractions, and the most active fraction Fr-2 (IC₅₀ = 2.5–5 µg/mL) was fractionated by C₁₈ SPE using 70% aqueous MeOH, 100% MeOH, and CH₂Cl₂ separately. The 100% MeOH fraction (IC₅₀ = 1.25–2.5 µg/mL) was divided into eight subfractions by silica-gel column chromatography (hexanes/EtOAc from 1:1 to 3:7 to 1:9, and a final elution with MeOH). The third subfraction (IC₅₀ < 1.25 µg/mL) was subjected to C₁₈ HPLC with a solvent gradient of H₂O/CH₂CN, 20:80 to 10:90 from 0.01 to 10 min, to 0.100 from 10 to 15 min, and ending with 100% CH₂CN to 30 min, furnishing compound 1 (tᵣ = 202 min, 1.1 mg, IC₅₀ = 2.98 µg/mL) and an active fraction (tᵣ = 208 min). Further purification of the active fraction by diol PTLC (hexanes/EtOAc = 4:6) yielded 3 (1.4 mg, IC₅₀ = 1.93 µg/mL) and 10 (0.4 mg, IC₅₀ = 5.74 µg/mL). The fourth subfraction from silica-gel column chromatography was subjected to C₁₈ HPLC and with a solvent gradient of H₂O/CH₂CN, 30:70–20:80 from 0.01 to 15 min, to 0:95 from 15 to 35 min, to 0:100 from 35 to 50 min, and ending with 100% CH₂CN to 60 min. Purification by diol PTLC (hexanes/EtOAc = 4:6) of the mixtures with tᵣ = 37.5 and 40.2 min obtained from this process gave compounds 5 (0.9 mg, IC₅₀ = 3.55 µg/mL), 11 (3.1 mg, IC₅₀ = 20 µg/mL), 2 (1.3 mg, IC₅₀ = 3.20 µg/mL) and 9 (0.5 mg, IC₅₀ = 6.57 µg/mL). The fifth subfraction from silica-gel column chromatography was further purified by C₁₈ HPLC by using the same profile as the fourth subfraction, giving pure compounds 8 (4.2 mg, tᵣ = 38 min, IC₅₀ = 13.6 µg/mL) and 6 (9.6 mg, tᵣ = 40.8 min, IC₅₀ = 1.58 µg/mL).

The hexanes fraction was subjected to Sephadex LH-20 gel chromatography (CH₂Cl₂/MeOH = 1:1) to give three fractions, and the most active fraction Fr-2 (IC₅₀ 2.5–5 µg/mL) was fractionated by preparative C₁₈ HPLC with a solvent gradient of H₂O/CH₂CN, 30:70–0:100 from 0.01 to 30 min, and ending with 100% CH₂CN to 45 min. This process yielded compound 7 (1.6 mg, tᵣ = 39 min, IC₅₀ = 2.50 µg/mL) and an active fraction (tᵣ = 32.5 min). Further purification of the fraction by diol HPLC with a solvent gradient of
was then analyzed by \(^1\)H NMR spectroscopy. The S-MPA esters of the S/6-dihydro-\(\alpha\)-pyrones were prepared from S-MPA chloride by the same method.

**Preparation of Bicyclic Tetrahydropyrones.** Compound 2 (0.2 mg) was dissolved in a mixture of MeOH (2 mL), EtOAc (0.5 mL), and hexanes (0.5 mL). Enough silica-gel powder was added to the solution to absorb it, and the mixture was kept at room temperature for 3 h. The resulting damp powder was eluted with MeOH, and the eluate concentrated on a rotavapor. The product was identified as 9 by \(^1\)H NMR spectroscopy. Compounds 3 and 5 (each 0.2 mg) were also treated separately and worked up under the same conditions. The products were identified as 10 and 11, respectively, by \(^1\)H NMR spectroscopy.

**Antiproliferative Bioassay.** The A2780 ovarian cancer cell line antiproliferative bioassay was performed at Virginia Tech as previously reported.\(^{32,33}\) The A2780 cell line is a drug-sensitive ovarian cancer cell line.\(^9\) Paclitaxel was used as the positive control; it had an IC\(_{50}\) value of 73 ± 15 nM.

**Antimalarial Bioassay.** The effect of each compound on parasite growth of the Dd2 strain of P. falciparum was measured in a 72 h treatment using the malaria SYBR green I-based fluorescence assay as described previously.\(^{35,36}\) Artemisinin was used as the positive control; it had an IC\(_{50}\) value of 7 ± 1 nM.

## ASSOCIATED CONTENT

### Supporting Information

\(^1\)H NMR spectra of compounds 1–11, Figures S1 (\(\Delta\delta(\mathrm{H}) = \delta_\mathrm{S} - \delta_\mathrm{R}\) data of R- and S-MPA derivatives), S2 (EI-MS fragmentations of the DMDS adducts), and S3 (ESI-MS fragmentations of compound S). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00187.

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### Notes

The authors declare no competing financial interest.

### Dedication

Dedicated to Professor Iwao Ojima in honor of his 70th birthday.

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