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THE SECONDARY METABOLITES OF AFF. *SAMADERA* SAC-2825: AN AUSTRALIAN SIMAROUBACEAE WITH UNUSUAL CHEMISTRY

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Key Word Index—SAC-2825 (aff. *Samadera bidwillii*); Simaroubaceae; Rutales; quassinoids; limonoids; 4-quinolone alkaloids; lignans; chemosystematics.

Abstract—The phytochemical analysis of two collections of a new species (SAC-2825), tentatively assigned as aff. *Samadera bidwillii* (Simaroubaceae), has yielded a limonoid (limonin), a quassinoid (2′-acetoxyglaucarubin), three alkaloids [2-(10′ξ-acetoxyundecanyl)-1-acetoxymethyl-4-quinolone, 1-methoxy-10-methyl-acridan-9-one and 1,8-dihydroxyacridan-9-one] and seven bicyclo-octane type lignans [(−)-sesamin, (−)-episesamin, fargesin, neofargesin, (−)-kobusin, (−)-epieudesmin and (−)-eudesmin]. The 4-quinolone alkaloid appears to be novel. The metabolites identified are collectively typical of the Rutales, but have a biosynthetic range never previously found together in a single species. Particularly noteworthy is the co-occurrence of limonoids and quassinoids in the same plant, which is currently unique to SAC-2825. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

A recently discovered species of Simaroubaceae from the Northern Territory of Australia, accession number SAC-2825, has been investigated. This taxon seems to be most nearly allied to an eastern Australian species which was originally described as *Hyptiandra bidwillii* J. D. Hooker and was subsequently treated as *Samadera bidwillii* (J. D. Hooker) Oliver, then as *Quassia bidwillii* (J. D. Hooker) Noteboom, and finally as *Simaba bidwillii* (J. D. Hooker) Feuillet. In our opinion, Hooker's species fits most comfortably in *Samadera*. Recently, Fernando *et al.* [1] have reported analysis of the nucleotide sequence for the plastid gene rbcL of plants from the SAC-2825 population and found it to be typical of the Simaroubaceae and therein most similar to that of *Samadera bidwillii* (as *Simaba bidwillii*). The morphology and classification of SAC-2825 will be dealt with in a future publication.

In this paper we wish to report the results of the phytochemical study, which have demonstrated SAC-2825 to be capable of the synthesis of an array of metabolites of great structural diversity and which reflect several of the major metabolic themes which are characteristic of the order Rutales [2–5]. Indeed, the complement of compounds found in SAC-2825 is such that the species contains compounds that had hitherto been unique to either the Simaroubaceae or to the Rutaceae.

RESULTS AND DISCUSSION

Two collections of SAC-2825 made from within a single population proved to be chemically identical. Compounds were separated from a bulked extract by vacuum liquid chromatography followed by preparative TLC or centrifugal TLC. The modified triterpene derivatives limonin (**1**) and 2′-acetoxyglaucarubin (**2**) were isolated and identified by direct comparison with samples that had previously been isolated in our laboratories.

A total of seven bicyclo-octane lignans were obtained. Of these, (−)-sesamin (**3**) and (−)-episesamin (**4**) and (−)-eudesmin (**5**) and (−)-epieudesmin (**6**) represent isomeric pairs differing only in the stereochemistry of one of the 2-aryl substituent. They were characterized primarily by comparison of their ^{1}H NMR data with that published [6]. A fifth lignan was identified as (−)-kobusin (**7**) [7], which has a stereochemistry comparable to that of sesamin and

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eudesmin. The final two lignans were inseparable. They contained the same aryl substituents as kobusin, but from the ^{1}H NMR spectrum appeared to have aryl stereochemistry comparable to episesamin and epieudesmin. The ^{1}H NMR data for one component was identical to that reported [8] for fargesin (**8**). The second component is presumed to be the lignan with the same stereochemistry as **8**, but with the substituents on the aryl groups reversed. We have called this second component neofargesin (**9**).

Two of the three alkaloids isolated had the spectral characteristics of acridones. One was identified as 1-methoxy-10-methylacridan-9-one (**10**) on the basis of NMR data and comparability of the melting point with that of the synthesized alkaloid [9]. The second, 1,8-dihydroxyacridan-9-one (**11**), has recently been isolated in our laboratory from *Boronia lanceolata* (Rutaceae) [10]. The HREI mass spectrum of the third alkaloid solved for a molecular ion $C_{25}H_{35}NO_5$. The ^{1}H NMR spectrum revealed the presence of four aromatic adjacent protons, one of which was deshielded (δ 8.33), indicative of placement *peri* to a carbonyl. A single olefinic proton at δ 6.16 could be assigned to the α-position of an α,β-unsaturated carbonyl, so requiring a 4-quinolone nucleus [10]. Other signals in the ^{1}H NMR spectrum were for two acetoxy methyls, a highly deshielded methylene (δ 6.09), an oxymethine, a methyl doublet and a methylene envelope.

A study of long-range H–C coupling interactions by means of the HMBC procedure [11] confirmed the presence of the 4-quinolone skeleton. The deshielded methylene protons revealed long-range coupling to C-2 and C-8a of the quinolone nucleus and to an acetoxy carbonyl. This required the presence of the unusual *N*-acetoxymethyl substituent that we had found previously in *Boronia* [10] and *Eriostemon* [12] species. From the requirement of the molecular formula this left the placement of an acetoxyl and an undecyl side chain at C-2 of the quinolone. The fact that the terminal methyl was a doublet indicated substitution at C-10′ of the side chain and on this basis the alkaloid was identified as 2-(10′ξ-acetoxyundecanyl)-1-acetoxymethyl-4-quinolone (**12**). The alkaloids showed very weak laevorotatory activity.

Chemotaxonomic significance

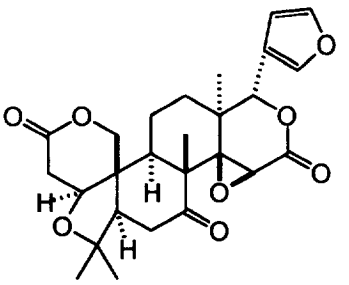
The major families of the Rutales (Rutaceae, Simaroubaceae and Meliaceae) are characterized by the occurrence of a wide range of secondary metabolites that are unique to the order or to individual families within the order [2, 5]. Thus, the Rutaceae produces an extensive range of alkaloids derived from anthranilic acid (2-quinolones, 4-quinolones, acridones, furoquinolines and ?carbazoles), tryptophan (canthinones and carbolines) and tyrosine (amides and isoquinolines), complex coumarins, polyoxygenated flavonoids and relatively simple tetranortriterpenoids (limonoids). The Meliaceae produce a vast array of highly complex limonoids while the Simaroubaceae yields mainly canthinone and carboline alkaloids and another group of modified triterpenes, the quassinoids. The limonoids and quassinoids represent two different biosynthetic themes arising from a tirucallane precursor (Scheme 1).

From what we currently know about the three families their metabolic profiles are remarkably consistent within these parameters. Whilst alkaloids typical of the Rutaceae have been recorded in both Meliaceae (carbazole) and Simaroubaceae (quinolone), they are very rare. Quassinoids have only been found in the Simaroubaceae, which as a family is free of limonoids except for the seemingly aberrant genus *Harrisonia*, which alone among genera traditionally assigned to the Simaroubaceae produces limonoids, but not quassinoids. Recent studies of DNA profiles support the contention that *Harrisonia* is not a typical Simaroubaceae (Morton, C., unpublished). To our knowledge the numerous isolations of quassinoids and limonoids recorded have never included their co-occurrence in a single species prior to this study.

Given the above observations then, SAC-2825 represents a metabolic anomaly. It yields both a classical simaroubaceous quassinoid, 2′-acetylglaucarubin, and a typically rutaceous limonoid, limonin. Furthermore, the acridone alkaloids it yields were previously unique to the Rutaceae as were 2-acylquinolin-4-ones, although simpler quinolones had been found in the Simaroubaceae [2]. It is not possible to make such clear statements about the lignans as their distribution has been much less well researched. However, from what is known [2, 13] it would appear that the bicyclo-octane type lignans are widespread in the Rutaceae, notably in *Zanthoxylum*, and not widely recorded from either Simaroubaceae or Meliaceae.

In identifying the chemosystematic significance of the secondary metabolic profile of SAC-2825 it is possible to dismiss any affinity to the extant Meliaceae, which are not known to produce limonoids with the limonin type of A-ring modification. Of the compounds found, 2′-acetylglaucarubin can be described as typically simaroubaceous, while limonin, the three alkaloids, and possibly even the lignans, are typically rutaceous. Limonin and alkaloids with the acridone skeleton, similar or identical to the compounds reported here, do occur in Australian taxa of the Rutaceae, although it is unclear that this has any systematic significance.

The Rutaceae and Simaroubaceae both produce carboline and canthinone alkaloids and it is tempting to think of the two families as dividing into separate evolutionary lines subsequent to the production of these alkaloid types, with the Rutaceae then going on to proliferate a wide range of alkaloids and limonin-type limonoids while the Simaroubaceae produced primarily quassinoids and a more limited array of alkaloids. If that is the case then SAC-2825 could be regarded as presenting a combined chemistry which could have existed in the primitive Rutales prior to that metabolic separation becoming established.



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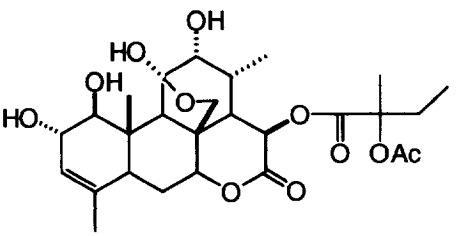

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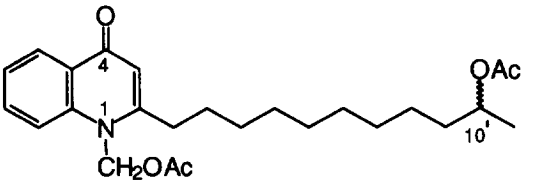

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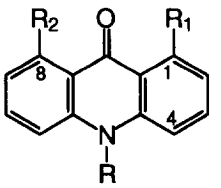


10 R = Me, R_1 = OMe, R_2 = H

11 R = H, R_1 = R_2 = OH

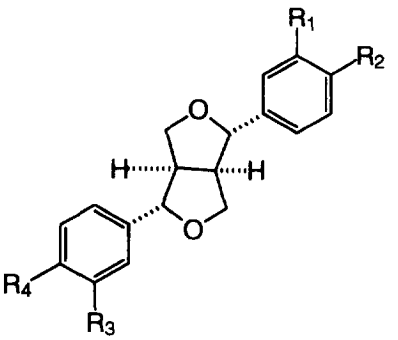
EXPERIMENTAL

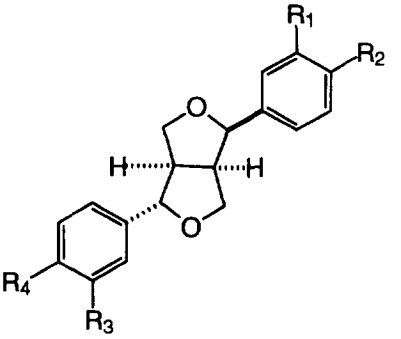
General. Mps: uncorr. UV: MeOH. IR: KBr disc. EIMS: AEI-MS 902 double-focusing spectrometer, direct probe insert at 110°, 70 eV. NMR spectra: Brukker AMX400 instrument calibrated to the solvent peak. 2D experiments used standard Brukker microprograms.

Collection details. The voucher SAC-2825, collected in the Northern Territory, has been deposited at The Australian National Herbarium, Canberra. Details of ecology of the plant will be published elsewhere.

Extraction. Two collections from the SAC-2825 population were extracted separately using a Soxhlet apparatus with, first, petrol, then $CHCl_3$. Sample 1 (60 g dry wt) yielded 700 mg petrol solubles and 1.2 g $CHCl_3$ solubles. Sample 2 (380 g), treated identically, yielded 4 g petrol solubles and 7.3 g $CHCl_3$ solubles.

Isolation of compounds. Preliminary examination of all extracts using TLC (silica gel) showed each to contain the same range of compounds, so the extracts were bulked for subsequent sepn of compounds. The combined extracts (10 g) were subjected to sepn by VLC over silica gel as previously described [14].





3 $R_1R_2 = OCH_2O$ $R_3R_4=OCH_2O$ **4** $R_1R_2 = OCH_2O$ $R_3R_4=OCH_2O$

5 $R_1=R_2=R_3=R_4=OMe$ **6** $R_1=R_2=R_3=R_4=OMe$

7 $R_1=R_2=OMe$ $R_3R_4=OCH_2O$ **8** $R_1=R_2=OMe$ $R_3R_4=OCH_2O$

9 $R_1R_2=OCH_2O$ $R_3R_4=OMe$

Preliminary sepn of compounds was achieved by elution of the VLC column with mixt. of petrol and EtOAc in which polarity was slowly increased by increasing the proportion of EtOAc.

The eluate with 40% EtOAc contained 2 compounds. These were subsequently sepd by centrifugal TLC (CTLC) on silica gel using a Chromatotron (Harrison Research model 7924). Elution with $CHCl_3$ gave one compound and $CHCl_3$ with 5% MeOH the 2nd compound. Each was then further purified by prep. TLC using silica gel with multiple elution by $CHCl_3$ containing 2% MeOH. This first yielded **3** (9 mg) and then **4** (8 mg).

The 50% EtOAc fr. from VLC yielded a mixt. of 6 compounds. These were further sepd into 3 frs by CTLC, eluting with $CHCl_3$ (fr. a), $CHCl_3$ with 5% MeOH (fr. b), and $CHCl_3$ with 15% MeOH (fr. c). Multiple prep. TLC of fr. a with petrol containing 20% $CHCl_3$ yielded **12** (60 mg) and **10** (6 mg). Fr. b was similarly sepd into two compounds by multiple elution prep. TLC with $CHCl_3$ containing 5% MeOH to yield an inseparable mixt. of **8** and **9** (23 mg) and **7** (12 mg). Multiple elution prep. TLC of fr. c with 15% MeOH in $CHCl_3$ gave **6** (51 mg) and **5** (29 mg).

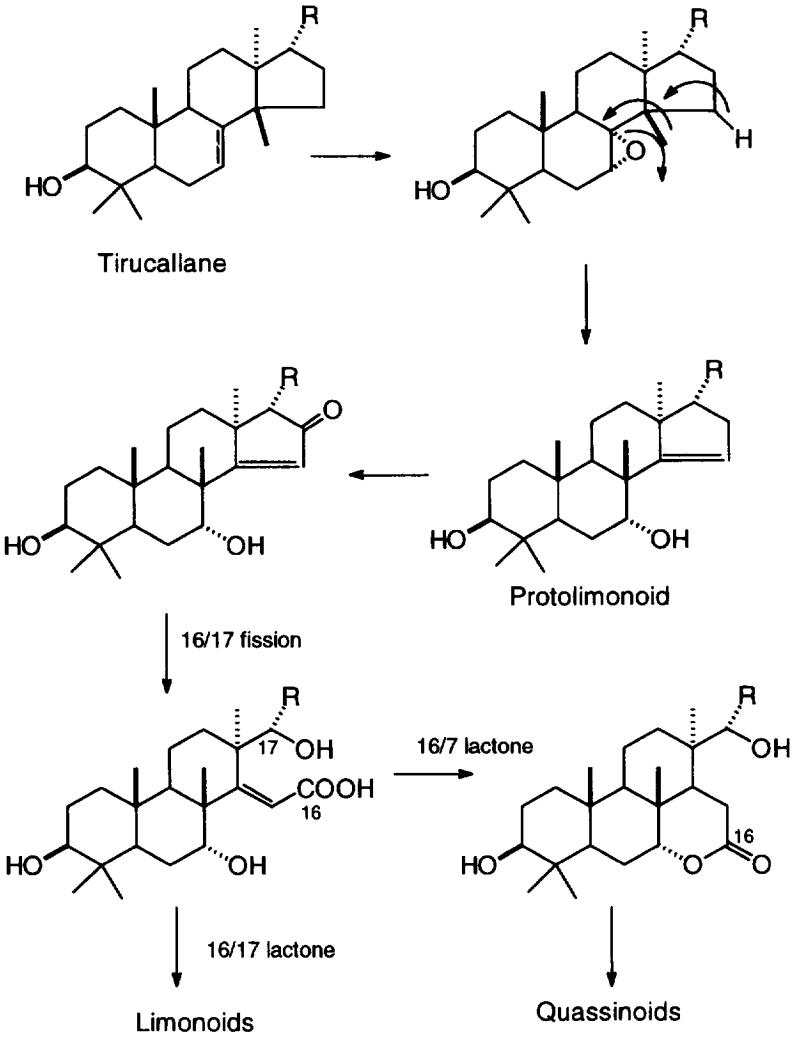
On concn of the 60% EtOAc fr. from VLC, **11** (5 mg) crystallized out. The supernatant was subjected to prep. TLC, eluting with toluene–EtOAc (1:1) to give **1** (10 mg). The more polar components from prep. TLC were re-chromatographed using $CHCl_3$–MeOH (9:1) to give **2** (6 mg).

Limonin (**1**). Recrystallized as cubes from MeOH, mp 296–298° (lit. [15]. 298°). HREIMS: found: $[M]^+$ 470.1902; $C_{26}H_{30}O_8$ requires 470.1941. Identical physical and chemical properties to a sample previously isolated in our laboratory from *Tetradium glabrifolium* [16].

2′-Acetoxyglaucarubin (**2**). Recrystallized as needles from Me_2CO mp 242–244° (lit. [17] 243–246°). HREIMS: found: $[M]^+$ 520.2320; $C_{27}H_{36}O_{10}$ requires 520.2308. Identical physical and chemical properties to a sample previously isolated in our laboratory from *Odyendyea gabonensis* Pierre [18].

2-{10′ξ-Acetoxyundecanyl}-1-acetoxymethyl-4-quinolone (**12**). Dark yellow oil. $[\alpha]_D - 1$ (*c* 0.45, MeOH). HREIMS: found: $[M]^+$ 429.2526; $C_{25}H_{35}NO_5$ requires 429.2515, with major fragments at 429 (1%), 387 (2%), 314 (13%), 298 (26%), 272 (14%), 258 (17%), 245 (21%), 231 (100%), 189 (38%), 159 (85%), 77 (15%). IR ν_{max}^{KBr} cm^{-1}: 3068, 2930, 2855, 1738, 1633, 1474, 1247, 1021, 960, 608. ^1H NMR (400 MHz, $CDCl_3$): δ 8.33 (1H, *dd*, $J = 8.0$, 1.7 Hz, H-5), 7.60 (1H, *td*, $J = 7.9$, 1.7 Hz, H-7), 7.46 (1H, *br d*, $J = 7.9$ Hz, H-8), 7.33 (1H, *td*, $J = 7.9$, 0.7 Hz, H-6), 6.16 (1H, *s*, H-3), 6.09 (2H, *s*, N-CH_2-O-), 4.82 (1H, *m*, H-10′), 2.71 (2H, *t*, $J = 6.6$ Hz, CH_2-1'), 2.09 (3H, *s*, Me-CO-), 1.97 (3H, *s*, Me-CO-), 1.65 (2H, *m*, CH_2-2′), 1.40–1.20 (14H, *m*, CH_2-3′–CH_2-9′), 1.14 (3H, *d*, $J = 6.2$ Hz, Me-11′). ^{13}C NMR (100 MHz, $CDCl_3$): δ *s* at 178.4 (C-4), 170.8, 170.0 (2 × Me-CO), 154.4 (C-2), 141.3 (C-8a), 126.3 (C-4a), *d* at 132.6 (C-7), 126.7 (C-5), 124.0 (C-6), 115.5 (C-8), 111.8 (C-3), 71.0 (C-10′), *t* at 68.8 (N-CH_2-O) 33.5 (C-1′), 28.6 (C-2′), 29.5–28.8 (C-3′–C-9′), *q* at 21.4, 20.8 (2 × Me-CO), 20.0 (C-11′).

1-Methoxy-10-methylacridan-9-one (**10**). Yellow, amorphous solid, mp 162–164° (lit. [9] 164°). HREIMS: found: $[M]^+$ 239.0939; $C_{15}H_{13}NO_2$ requires 239.0948, with major fragments at 239 (100%), 222



Scheme 1.

(39%), 210 (39%), 193 (23%), 119 (12%), 90 (10%), 77 (10%). IR ν_{max}^{KBr} cm^{-1}: 3088, 3041, 2988, 2955, 1732, 1630, 1603, 1563, 1491, 1248, 1204, 1085, 770. ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (1H, *dd*, J = 8.0, 1.6 Hz, H-8), 7.66 (1H, *td*, J = 7.0, 1.7 Hz, H-6), 7.59 (1H, *br t*, J = 8.5 Hz, H-3), 7.43 (1H, *br d*, J = 8.6 Hz, H-5), 7.26 (1H, *td*, J = 7.9, 0.7 Hz, H-7), 7.05 (1H, *br d*, J = 8.7 Hz, H-3), 6.72 (1H, *br d*, J = 8.2 Hz, H-2), 4.03 (3H, *s*, OMe-1′), 3.84 (3H, *s*, *N*-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ *s* at 178.2 (C-9), 161.9 (C-1), 145.6 (C-10a), 142.1 (C-4a), 124.8 (C-8a), 113.6 (C-1a), *d* at 134.0 (C-3), 133.3 (C-6), 128.5 (C-8), 121.5 (C-7), 114.4 (C-5), 107.1 (C-4), 103.0 (C-2), *q* at 56.5 (OMe-1), 35.0 (*N*-Me).

1,8-*Dihydroxyacridan-9-one* (**11**). Yellow, amorphous, mp 248–250° (lit. [10] 250°). HREIMS: found $[M]^+$ 227.0580; $C_{13}H_9NO_3$ requires 227.0582. Spectral data was in accord with that published previously [10].

(−)-*Sesamin* (**3**). Gum, $[\alpha]_D$ −35 (*c* 0.12, $CHCl_3$). Found $[M]^+$ 354.1110; $C_{20}H_{18}O_6$ requires 354.1103. NMR data in agreement with that published [6].

(−)-*Episesamin* (**4**). Gum, $[\alpha]_D$ −13 (*c* 0.08, $CHCl_3$). Found $[M]^+$ 354.1110; $C_{20}H_{18}O_6$ requires 354.1103. NMR data in agreement with that published [6].

Fargesin (**8**) *and neofargesin* (**9**). Brown oil, $[\alpha]_D$ −65 (*c* 0.2, $CHCl_3$). Found: $[M]^+$ 370.1411; $C_{21}H_{22}O_6$ requires 370.1416. ^1H NMR (400 MHz, $CDCl_3$) δ (signals comparable in the two compounds): 2.88 (2H, *m*, H-1), 3.31 (2H, *m*, H-5), 3.35, 3.80 (4H, 2 × *m*, H_2-4), 3.80 (2H, *m*, H-8), 4.12 (2H, *d*, J = 9.4 Hz, H-8), 4.43 (2H, *d*, J = 7.1 Hz, H-6), 6.70–6.95 (aromatic); signals attributable to only one compound, 4.87 (1H, *d*, J = 5.4 Hz, H-2), 4.84 (1H, *d*, J = 5.4 Hz, H-2), 3.87, 3.88, 3.90, 3.91 (4 × OMe), 5.94, 5.95 (2 × O-CH_2-O).

(−)-*Kobusin* (**7**). Oil, $[\alpha]_D$ −21 (*c* 0.38, $CHCl_3$). Found $[M]^+$ 370.1412; $C_{21}H_{22}O_6$ requires 370.1416. NMR data in agreement with that published [7].

(−)-*Epieudesmin* (**6**). Oil, $[\alpha]_D$ −101 (*c* 0.15, $CHCl_3$). Found $[M]^+$ 386.1725; $C_{22}H_{26}O_6$ requires 386.1730. NMR data in agreement with that published [6].

(−)-*Eudesmin* (**5**). Gum, $[\alpha]_D$ −15 (*c* 0.1, $CHCl_3$). Found $[M]^+$ 386.1721; $C_{22}H_{26}O_6$ requires 396.1730. NMR data in agreement with that published [6].

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