REVISION OF THE STRUCTURES OF THE BENZO[*a*]NAPHTHACENE QUINONE METABOLITES G-2N AND G-2A FROM BACTERIA OF THE GENUS *Frankia*

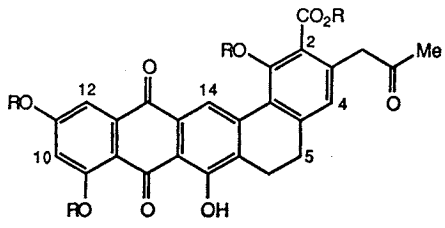
Sir:

The rapidly expanding group of bacterial benzo[*a*]naphthacene quinone metabolites from actinomycetes includes KS-619-1 (**1**), a potent inhibitor of calcium- and calmodulin-dependent cyclic nucleotide phosphodiesterase from *Streptomyces californicus*,[1,2] the six members of the SF2446 complex from *Streptomyces* sp. SF2446 which are active against mycoplasmas and Gram-positive bacteria,[3,4] and the antifungal and antibacterial benanomicins A and B (**3**) from an unidentified *Actinomycete* sp. MH193-16F4.[5] These compounds are all derivatives of 5,6-dihydrobenzo[*a*]naphthacene quinones, and in particular carry 3-alkyl-2-carboxy-1-hydroxy substituents in the angular E ring. Structural analysis of these metabolites indicates a biosynthetic origin from a single acetate-derived polyketide chain, which starts at the alkyl group in the E ring and terminates at the adjacent carboxyl group. In contrast, the first natural products with this ring system to be isolated, G-2N and G-2A from various actinomycetes of the genus *Frankia*, were assigned the 2-alkyl-4-hydroxy and 2-alkyl-10- or 11-carboxy-4-hydroxy structures (**4**) and (**5**).[6] GERBER and LECHEVALIER[6] noted that G-2N and G-2A appeared to be products of polyketide biosynthesis, and suggested that two polyketide chains were probably involved in view of the location of the methyl and carboxyl groups in the E and A rings.

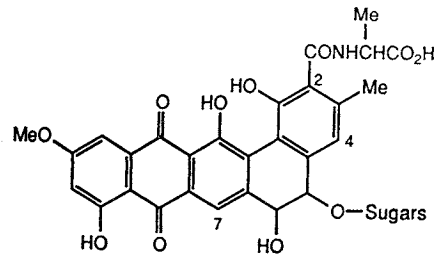
Reconsideration of the published spectroscopic evidence for the *Frankia* compounds G-2N and G-2A now leads to the revised structures (**6**) and (**7**), which are structurally and biosynthetically closely related to the other members of the benzo[*a*]naphthacene quinone group.

The substitution patterns of the A and E rings in G-2N are described by the two two-spin systems in the ^{1}H NMR spectrum; the slightly broadened singlets at δ 6.70 and 6.68, and the *m*-coupled (J=2.5 Hz) doublets at δ 7.16 and 6.61 (in DMSO-d_6). In proposing structure (**4**) for G-2N, GERBER and LECHEVALIER[6] assigned these systems to the similar *o*-related protons 10-H and 11-H of the A ring, and to 1-H and 3-H of the E ring, respectively. 10-H and 11-H, however, would be expected to resemble in chemical shift 2-H and 3-H of 1,4-dihydroxy-anthraquinone, which resonate at δ 7.30,[7] while the weak *m*-coupling between 1-H and 3-H might be expected to be obscured by benzylic coupling[8] to the 2-methyl and 5-methylene protons. These difficulties are overcome in the revised structure (**6**) for G-2N, where the former system now represents 2-H and 4-H of the E ring and the latter 10-H and 12-H of the A ring. As seen in the Table 1, the A ring protons in G-2N now match closely in chemical shift and coupling constant those in KS-619-1 (**1**), as do the corresponding protons in the trimethyl ether of G-2N (**8**) and the trimethyl ether methyl ester of KS-619-1 (**2**). The chemical shifts of 5-H_2 and 6-H_2 in these two pairs of compounds also agree. The distinctive deshielding of the isolated aromatic proton singlet at δ 8.81 in G-2N locates it at C-14, as discussed previously;[6] the alternative location at C-7 is significantly more shielded, as seen in the δ 8.05 (in DMSO-d_6) shift of the 7-H singlet in the benanomicins (**3**).[5] Differences in the shifts of 14-H between G-2N and KS-619-1, and also between their methylation products, are due to the presence of the additional carboxy or carbomethoxy substituent at C-2 in KS-619-1 (**1**) and its derivative (**2**). Acetylation of G-2N afforded[6] a tetraacetate, in which the four protons of the two two-spin systems were shifted substantially downfield. As expected for structure (**6**), the 10-H and 12-H protons in the *m*-dihydroxylated A ring experienced the largest acylation shifts to δ 7.23 and 7.95,[9] respectively, while 2-H and 4-H in the monohydroxylated E ring moved only to δ 6.9~7.0 (in $CDCl_3$).[10,11]

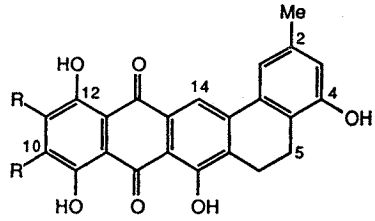
These NMR data relate the substitution in the A, B, C and D rings of G-2N to the corresponding substitution in KS-619-1 (**1**). The additional carboxyl group and side-chain acetyl unit in KS-619-1 will affect the resonance position of 4-H, thus nullifying direct comparison of chemical shifts of the E ring protons in the two compounds. The close similarity of chemical shifts of the two E ring protons in G-2N and in its methylation and acetylation products, however, establishes that neither proton is at C-1, where it would be substantially deshielded by the aromatic C ring. The corresponding proton



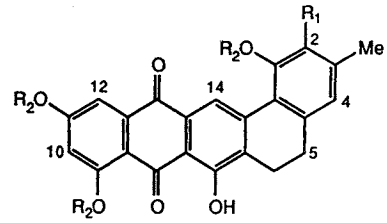
1 R=H
2 R=Me



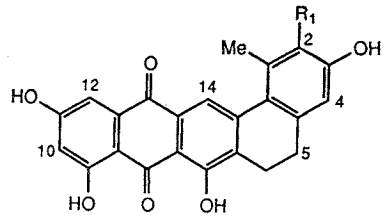
3



4 R=R=H
5 R, R=H, COOH



6 R_1=R_2=H
7 R_1=COOH R_2=H
8 R_1=H R_2=Me



9 R_1=H
10 R_1=COOH

Table 1. Comparative ^{1}H NMR data of compounds **1**, **2**, **6** and **8**.[a]

Proton	1[b]	6[c]	2[b]	8[c]
2-H	—	6.70 br s[d]	—	6.73 br s
4-H	6.38 s	6.68 br s[d]	6.95 s	6.73 br s
5-H_2	~2.7 m	2.76 s	~2.8 m	2.75~2.89 m
6-H_2	~2.8 m	2.76 s	~3.0 m	2.75~2.89 m
10-H	6.58 d[f]	6.61 d[g]	6.81 d[g]	6.78 d[g]
12-H	7.15 d[f]	7.16 d[g]	7.51 d[g]	7.50 d[g]
14-H	9.08 s	8.81 s	8.77 s	8.72 s
7-OH			13.60 s	13.52 s
1-OCH_3	—	—	3.65 s	3.94[e]
9-OCH_3	—	—	4.05 s	4.03[e]
11-OCH_3	—	—	4.00 s	3.98[e]

[a] Data for **1** and **6** were recorded in DMSO-d_6, for **2** and **8** in $CDCl_3$.
[b] Recorded at 400 MHz by YASUZAWA *et al.*[2]
[c] Recorded at 80 or 100 MHz by GERBER and LECHEVALIER.[6]
[d,e] Assignments for signals in these groups may be interchanged.
[f] J=2.2 Hz.
[g] J=2.5 Hz.

in 9,10-dihydrophenanthrene, for example, resonates at δ 7.73, compared to the remaining three protons of the terminal ring which occur between δ 7.17~7.32 (in $CDCl_3$).[12] There are thus only two possible orientations, represented in structures (**6**) and (**9**), for the E ring substituents of G-2N. The distinction between these two structures for G-2N necessitates consideration of the structure of the co-metabolite G-2A.

From electronic, IR, ^1H NMR and mass spectrometry data the co-metabolite G-2A is closely related to G-2N and contains an additional carboxyl group.[6] ^1H NMR data were not reported for underivatised G-2A, thus precluding comparison with those for KS-619-1 (**1**). Since G-2A yields only a triacetate under mild conditions where G-2N afforded the tetraacetate, the carboxyl group was suggested to be adjacent to one of the hydroxyl groups.[6] More vigorous acetylation of G-2A gave a tetraacetate in which the resonances due to 5-H and 6-H (δ 2.79, br s), 10-H and 12-H (δ 7.50 and 7.90, each d, J= 2.5 Hz), and 14-H (δ 8.67, s) remained visible in the ^1H NMR spectrum (in DMSO-d_6), accompanied by an aromatic proton singlet at δ 7.3. The carboxyl group is therefore located adjacent to the hydroxyl group in the E ring. Furthermore, since both compounds G-2N and G-2A are clearly derived biosynthetically from acetate and probably *via* a single polyketide chain, the carboxyl and methyl groups representing the ends of the chain would be expected to be adjacent to each other after cyclization. This leads to the substitution patterns (**7**) or (**10**) for G-2A. Of these, structure (**7**) for G-2A is selected for two reasons. First, it accounts better for the difficulty in forming the tetraacetate of G-2A in contrast to that of G-2N; the hydroxyl group at C-1 in **7** is not only H-bonded to the additional carboxyl, but is now also severely sterically hindered. The environment of the hydroxyl at C-3 in the alternative orientation (**10**), on the other hand, resembles that in salicylic acid which acetylates relatively readily. Secondly, electronic absorption maxima reported for G-2A in ethanol at 301 and 463 nm, and in alkaline ethanol at 297, 345 and 540 nm, correspond to maxima at 302 and 470 nm and 298, 330 and 530† nm in published spectra of KS-619-1 (**1**) in methanol.[1] The two chromophores are clearly similar, small differences in wavelength possibly being caused by differences in spectral pH affecting the extent of ionization.

Accordingly, the *Frankia* metabolite G-2A has the structure 8,13-dioxo-3-methyl-5,6,8,13-tetrahydro-1,7,9,11-tetrahydroxybenzo[*a*]naphthacene-2-carboxylic acid (**7**). It is a dodecaketide related biosynthetically to the actinomycete metabolites of the SF2446 and benanomicin complexes, and particularly to the phosphodiesterase inhibitor KS-619-1 (**1**) which differs from G-2A only in having an additional acetate unit in its carbon skeleton. The *Frankia* metabolite G-2N (**6**) is the decarboxylation product of the acid G-2A. An examination of the biological activity of G-2A and G-2N would be of interest in view of the activity shown by the more complex representatives of the benzo[*a*]naphthacene quinone group.

† The value of 515 nm reported in Table 2 of MATSUDA and KASE[1] does not agree with the spectrum published in Fig. 4(B).

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