

## SESQUITERPENE LACTONES FROM *SQUAMOPAPPUS SKUTCHII*

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(Received 24 July 1987)

**Key Word Index**—*Squamopappus skutchii*, Asteraceae, Heliantheae, sesquiterpene lactones, eudesmanolide, guaianolides

**Abstract**—Chemical analysis of *Squamopappus skutchii* afforded a new eudesmanolide, chapinolin, and two new guaianolides, skutchinolide A and B. Their structures were elucidated by <sup>1</sup>H NMR and mass spectral methods.

### INTRODUCTION

In our biochemical systematic study of the tribe Heliantheae (Asteraceae) [1] we were interested to learn about the chemical constituents of *Squamopappus skutchii* since this taxon had recently been placed into three different subtribes. Originally classified by Blake as *Calea skutchii* of the subtribe Galinsogineae [2] it was later transferred to the subtribe Verbesiniae by Robinson [3], who expanded the genus *Podachaenium* to include this taxon as *P. skutchii*. Based on morphological grounds, Jansen *et al.* [4] recently transferred it to the subtribe Ecliptinae as the monotypic genus *Squamopappus skutchii*.

We wish to report here the structures of three new sesquiterpene lactones, two guaianolides and a eudesmanolide, all of which were present in two populations of *S. skutchii* from Guatemala. In addition, the sesquiterpene  $\beta$ -eudesmol (**1**) was found in one of the populations and was characterized by comparison of its <sup>1</sup>H NMR and mass spectral data with those described in the literature [5]. The structures of the three new compounds were established by spectroscopic techniques (NMR and MS) as described below.

### RESULTS AND DISCUSSION

Chapinolin (**2**)  $C_{20}H_{26}O_5$ , was a gum with IR spectral bands at 1760 and 1715  $\text{cm}^{-1}$  indicating the presence of a  $\gamma$ -lactone and an ester group, respectively. The presence of an  $\alpha$ -methylene- $\gamma$ -lactone moiety was confirmed by the appearance of typical downfield doublets in the <sup>1</sup>H NMR spectrum at  $\delta$  6.12 (H-13a) and 5.54 (H-13b) and a multiplet at 2.89 (H-7). The ester group was identified as an angelate based on diagnostic <sup>1</sup>H NMR signals (Table 1) and a typical mass spectral pattern with a base peak at *m/z* 83 (**B**<sup>1</sup>) and another strong peak at *m/z* 55 (**B**<sup>2</sup>).

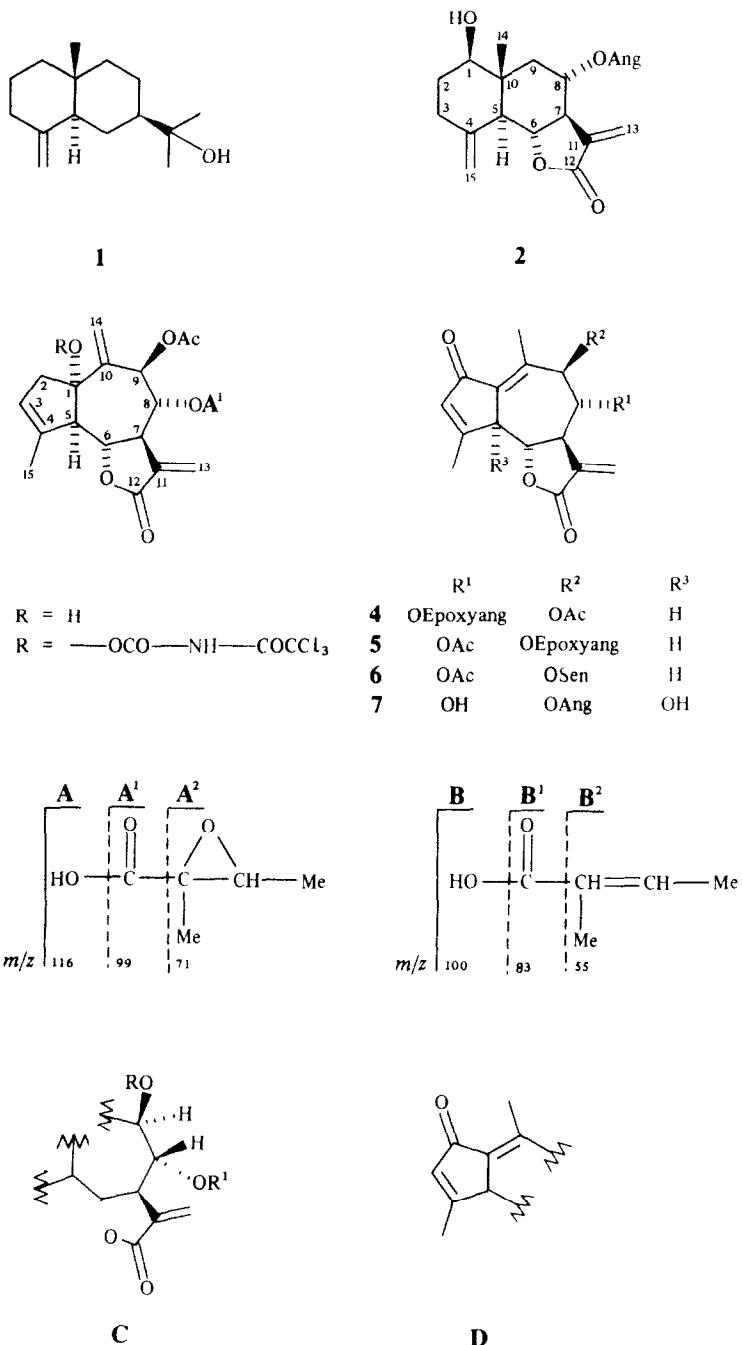
A three-proton singlet at  $\delta$  0.89 indicated the presence of an angular methyl group, which suggested a eudesmanolide skeleton for chapinolin. The rest of the signals in the <sup>1</sup>H NMR spectrum were assigned by extensive decoupling experiments, the assignments being summarized in Table 1.

Irradiation of the signal at  $\delta$  2.89 (H-7) collapsed the

doublet of a doublet at  $\delta$  4.13 to a doublet, and the threefold doublet at  $\delta$  5.29 changed to a doublet of a doublet. On the basis of chemical shifts, the signals at  $\delta$  4.13 and 5.29 were assigned to the lactonic proton and the proton on the carbon bearing the ester substituent, respectively. Saturation of the signal at  $\delta$  4.13 (H-6) affected the multiplet at  $\delta$  2.89 (H-7) and the broad doublet at  $\delta$  2.22 (H-5) collapsed to a singlet. The above decouplings allowed the following assignments.  $\delta$  2.22 (H-5 $\alpha$ ), 4.13 (H-6 $\beta$ ), 2.89 (H-7 $\alpha$ ) and 5.29 (H-8 $\beta$ ). The magnitude of the coupling constants ( $J_{5,6} = J_{6,7} = J_{7,8} = 11.0$  Hz) was in full agreement with an antiperiplanar disposition of H-5/H-6, H-6/H-7, and H-7/H-8. Assuming that H-7 is  $\alpha$  as in all lactones from higher plants [6], H-5 should also be  $\alpha$ - and H-6 and H-8 be  $\beta$ -oriented. The two broadened one-proton singlets at  $\delta$  5.01 and 4.88 sharpened upon irradiation of H-5, indicating allylic coupling of this proton with the exocyclic methylene protons (H-15a and H-15b). On the basis of chemical shift arguments, the doublet of a doublet at  $\delta$  3.52 was assigned to the proton at a carbon bearing the hydroxyl group. Its multiplicity suggested coupling to two protons. The only positions allowing for this arrangement are C-1 and C-3. Attachment of the hydroxyl group at C-3 was excluded on the basis that H-3 would have shifted farther downfield near 4 ppm due to its allylic position. Also, a more complex signal would have been expected for H-3 due to its allylic couplings to the two C-15 protons. In contrast, the NMR data were in good agreement with the hydroxyl group being attached to C-1. Based on the magnitude of the coupling constants of H-1 ( $J_{1,2a} = 11.0$  Hz,  $J_{1,2b} = 5.0$  Hz), the orientation of H-1 should be axial or  $\alpha$ . Comparison of the <sup>1</sup>H NMR spectrum with data reported for the 8-desangeloyloxy compound reynosin [7], support stereostructure **2** for chapinolin.

Skutchiolide A (**3a**) was a gum with a molecular formula of  $C_{22}H_{26}O_8$ . The <sup>1</sup>H NMR spectrum of **3a** displayed two one-proton doublets at  $\delta$  6.22 (H-13a) and 5.67 (H-13b), and a multiplet at 3.45 (H-7) which are characteristic of an  $\alpha$ -methylene- $\gamma$ -lactone. An IR band at 1770  $\text{cm}^{-1}$  confirmed the presence of a  $\gamma$ -lactone moiety. The IR spectrum showed two further bands at 1710  $\text{cm}^{-1}$  and 1720  $\text{cm}^{-1}$  which suggested the presence of two ester groups. A three-proton singlet at  $\delta$  2.03 in the <sup>1</sup>H NMR spectrum and mass spectral peaks at *m/z* 358 (M - AcOH) and 43 (Ac) indicated the presence of an acetate side chain. The other ester group was identified as

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an epoxylanolate moiety based on diagnostic <sup>1</sup>H NMR signals at  $\delta$  3.08 (quartet, H-3'), a three-proton singlet at 1.57 (C-2'-Me) and a three-proton doublet at 1.35 (C-3'-Me). Typical mass spectral peaks at *m/z* 116 (A), 99 (A<sup>1</sup>) and 71 (A<sup>2</sup>) supported the NMR assignments. Detailed <sup>1</sup>H NMR double resonance experiments allowed the structural assignments of skutchinolide A (Table 1).

Irradiation of the multiplet at  $\delta$  3.45 (H-7) collapsed the two doublets at  $\delta$  6.22 (H-13a) and  $\delta$  5.67 (H-13b) to singlets, and changed the two doublets of doublets at  $\delta$  3.87 and 5.31 into doublets. On the basis of its chemical shift, the signal at  $\delta$  3.87 was assigned to the lactonic proton (H-6). Saturation of the lactonic proton collapsed

the doublet at  $\delta$  2.85 to a broad singlet. Irradiation of the doublet of a doublet at  $\delta$  5.31 changed the doublet at 5.65 to a singlet. Based on these couplings, the chemical shifts were assigned as follows:  $\delta$  2.85 (H-5), 3.87 (H-6), 5.31 (H-8) and 5.65 (H-9). The magnitude of the coupling constants ( $J_{5,6} = 9.6$  Hz,  $J_{6,7} = 10.5$  Hz,  $J_{7,8} = 10.0$  Hz and  $J_{8,9} = 9.3$  Hz), and taking into account that H-7 is  $\alpha$  [6], the protons from C-5 to C-9 must have antiperiplanar arrangements, suggesting a stereochemistry depicted in the partial structure C. The two one-proton singlets at  $\delta$  5.41 and 5.29 are typical of an exocyclic methylene group. Since both signals sharpened upon irradiation of H-9 the allylic coupling indicated that the olefinic bond

Table 1  $^1\text{H}$  NMR spectral data of chapinolin (2), skutchiolides A (3a) and B (4) and derivative 3b\*

H	2	3a	3b [ $\delta_{3b} - \delta_{3a}$ ]	4
1	3.52 dd (11.0, 5.0)	—	—	—
2a	2.29 m	2.90 br d (16.5)	3.10 [0.20]	—
2b	1.43 m	2.53 br d (16.5)	3.10 [0.57]	—
3	†	5.52 br d	5.58 [0.06]	6.21 br s†
5	2.22 br d (11.0)	2.85 br d (9.5)	3.20 [0.35]	3.62 m
6	4.13 dd (11.0, 11.0)	3.87 dd (10.5, 9.5)	4.05 [0.17]	3.64 m†
7	2.89 dddd (11.0, 11.0, 3.0, 3.0)	3.45 dddd (10.5, 10.0, 3.5, 3.0)	3.62 [0.20]	3.48 m
8	5.29 ddd (11.0, 11.0; 4.0)	5.31 dd (10.0, 9.3)	5.20 [0.11]	5.29 m
9a	2.54 dd (13.0, 4.0)	5.65 d (9.3)	5.63 [0.02]	5.63 d (~ 10)†
9b	2.02†	—	—	—
13a	6.12 d (3.0)	6.22 d (3.5)	6.31 [0.09]	6.19 d (3.0)
13b	5.54 d (3.0)	5.67 d (3.0)	5.76 [0.09]	5.60 d (2.5)
14a	—	5.41 br d	5.70 [0.29]	—
14b	—	5.29 br d	5.56 [0.27]	—
C-10-Me	0.89 s	—	—	2.26 s
15a	5.01 br s	—	—	—
15b	4.88 br s	—	—	—
C-4-Me	—	1.86 br d	1.90 [0.04]	2.32 br s
MeCO	—	2.03 s	2.04 [0.01]	2.13 s
3'	6.17 br d	3.08 q (5.5)	3.10 [0.02]	3.11 q (5.7)
C-2'-Me	1.88 q	1.57 s	1.54 [0.03]	1.57 s
C-3'-Me	1.99 dq	1.35 d (5.5)	1.37 [0.02]	1.41 d (5.7)
NH	—	—	8.5	—

\* Spectra were obtained at 200 MHz in  $\text{CDCl}_3$ . Multiplets are given by the usual symbols, chemical shifts ( $\delta$ ) are recorded in ppm relative to TMS. Coupling constants ( $J$ ) or line separations in Hz are given in parenthesis

† Obscured by other signals, therefore, couplings could not be accurately determined

must be between C-10 and C-14. This finding also supported a guaianolide skeleton for skutchiolide A. When the vinyl proton signal at  $\delta$  5.52 (H-3) was irradiated, the doublets of doublets at  $\delta$  2.90 and 2.53 (H-2) resolved into doublets with a coupling of 16.5 Hz each, suggesting a geminal relationship of the two latter protons

Furthermore, the three-proton singlet at  $\delta$  1.86 (C-4-Me) also sharpened, indicating allylic coupling between an olefinic methyl group and the vinyl proton at  $\delta$  5.52 (H-3). The absence of further couplings, other than the geminal coupling ( $J = 16.5$  Hz) for the signals at  $\delta$  2.90 (H-2a) and 2.53 (H-2b), suggested that this methylene group must be placed next to a quaternary carbon. Since an IR band at  $3410 \text{ cm}^{-1}$  indicated the presence of a hydroxyl group, it had to be attached to C-1. This was further confirmed by *in situ* preparation of the trichloroacetylcarbamate derivative (3b). The appearance of a one-proton signal at  $\delta$  8.5 in the  $^1\text{H}$  NMR spectrum of 3b was evidence for the formation of one carbamate moiety, indicating the presence of one hydroxyl group in 3a. The chemical shift difference of the H-2 triplet at  $\delta$  2.53 in 3a to 3.10 in 3b ( $\Delta\delta = 0.57$ ) was in agreement with an expected  $\beta$ -effect [8]. In addition, H-5 shifted from  $\delta$  2.85 in 3a to 3.20 in 3b ( $\Delta\delta = 0.35$ ). Since H-5 had been assigned an  $\alpha$ -orientation based on its large  $J$ -value with H-6, the above shown  $\beta$ -effect upon H-5 indicated that the hydroxyl group of C-1 must also be  $\alpha$ -oriented. This established the skeletal arrangement and stereochemistry of skutchiolide A as shown in structure 3a, exclusive of the site of attachment of the two ester groups. The attachment of the epoxyangelate and acetate moieties to C-8 and C-9, respectively, or the reverse arrangements, was

not resolved at this point. However, a tentative structural proposal will be presented together with the assignments of the skutchiolide B structure.

Skutchiolide B (4),  $\text{C}_{22}\text{H}_{24}\text{O}_8$ , was a gum with an IR band at  $1760 \text{ cm}^{-1}$  indicating a  $\gamma$ -lactone. This was confirmed by diagnostic  $^1\text{H}$  NMR signals: two one-proton doublets at  $\delta$  6.19 (H-13a) and  $\delta$  5.60 (H-13b) which were coupled to the multiplet at  $\delta$  3.48 (H-7). Two additional IR bands appeared at 1715 and  $1700 \text{ cm}^{-1}$  suggesting the presence of two ester groups. The identities of the ester side chains were established as acetate and epoxyangelate by comparison of their spectral data with those of the side chains of lactones 3a. Decoupling experiments allowed the assignment of the remaining  $^1\text{H}$  NMR signals (Table 1).

The presence of two three-proton singlets at  $\delta$  2.32 and 2.26 suggested two methyl groups attached to the  $\beta$ -carbons on an  $\alpha,\beta$ -unsaturated carbonyl moiety. Furthermore, lactone 4 exhibited IR bands at  $1670$ ,  $1650$  and  $1620 \text{ cm}^{-1}$ . The above  $^1\text{H}$  NMR and IR absorptions are typical of fragment D which can be found in other guaianolides [9].

Irradiation of the signal at  $\delta$  6.21 (H-3) caused sharpening of the methyl singlet at  $\delta$  2.32 (C-4-Me) indicating allylic coupling. Saturation of the H-7 signal collapsed the H-13a and H-13b doublets and affected the signals at  $\delta$  3.64 (H-6) and 5.29 (H-8). When the signal at  $\delta$  5.29 was irradiated the only changes observed were simplification of the H-7 multiplet and collapse of the obscured doublet at  $\delta$  5.63 into a singlet. The lactonic proton was coupled to H-7 as well as to a signal at  $\delta$  3.62, which was assigned to the proton at C-5. Spectral characteristics of skutchiol-

ide B compared closely with those of pumilin (7) [9], which has an additional  $\alpha$ -hydroxyl group at C-5. Based on the above finding, the guaianolide skeleton of skutchiolide B is that shown in structure 4

The location of the ester substituents in 3a and 4, acetate and epoxyangelate, were tentatively assigned by comparison of the  $^1\text{H}$  NMR spectral data with those of 17,18-epoxy-5-desoxypumilin-8-O-acetate (5) which has the same substituents as 3a and 4 and the senecioate analogue (6) [10]. The  $^1\text{H}$  NMR absorptions for H-7 in compounds 5 and 6 appear near  $\delta$  3.0 whereas lactone 4 shows a multiplet at 3.48. Also, in 5 and 6 the H-8 and H-9 signals differ significantly from those of skutchiolide A and B. Therefore, we tentatively assign the ester substituents in the two new guaianolides as shown in the respective structures 3a and 4.

The guaianolides found in *S skutchu* are distinctly different in their substitution pattern from those present in *Podachaenium* [11] and *Calea* [12] species. The main differences reside in the presence of ester substituents at C-9 of the *Squamopappus* guaianolides which are not present in the other two genera. This type of C-8, C-9 substitution pattern is typical for guaianolides from *Berlandiera* species [9, 13, 14] of the subtribe Engelmanninae [15], constituents of *Montanoa* [16] of the subtribe Montanoinae and guaianolides from *Balsamorhiza sagittata* [10] of the Ecliptinae. Therefore, the new chemical data support the removal of *S skutchu* from *Calea* and *Podachaenium*. The structural similarities of the *S skutchu* sesquiterpene lactones with those isolated from *B sagittata* support the proposal based on morphological findings that *S skutchu* be placed as a new monotypic genus in the subtribe Ecliptinae [4].

## EXPERIMENTAL

*Squamopappus skutchu* (S F Blake) R. Jansen, N. Harriman and L. Urbatsch was collected on 11 January 1977 in Totonicapan, Guatemala (L Urbatsch, No 3022, voucher deposited at LSU, USA). The air-dried ground material (1 kg) was extracted and worked-up as previously described [17], providing 21 g of crude terpenoid extract. Flash CC of the crude syrup with  $\text{CHCl}_3$ -EtOAc (7:3) provided 25 fractions of 100 ml each. Fractions 9, 10 (250 mg) were rechromatographed on a silica gel column  $\text{CHCl}_3$ -Me<sub>2</sub>CO mixtures of increasing polarity. Twenty 50 ml fractions were collected. Fractions 5, 6 were purified by prep TLC providing 7 mg of skutchiolide A (3a) and 11 mg of skutchiolide B (4). Fraction 7, after separation on prep TLC, yielded 8 mg of chapinolin (2).

A second population of *S skutchu* was collected on 12 January 1977 in Quetzaltenango, Guatemala (L Urbatsch, No 3023, voucher deposited at LSU, USA). The air-dried plant material (770 g) was ground and extracted and worked-up in the fashion described previously [17], providing 17 g of crude extract. Column chromatography of the crude terpenoid syrup with  $\text{CHCl}_3$ -MeCO mixtures of increasing polarity furnished 35 fractions of 100 ml each. Fractions 20-22 (570 mg) were combined and rechromatographed on a silica gel column with petrol- $\text{CHCl}_3$  and  $\text{CHCl}_3$ -Me<sub>2</sub>CO mixtures of increasing polarity. 75 fractions of 25 ml each were collected. Fractions 10-16 (125 mg) were combined and purified by prep TLC using a mixture of Et<sub>2</sub>O-petrol (2:3), providing 60 mg of  $\beta$ -eudesmol (1). Fractions 31-34 (53 mg) were combined and further separated by prep TLC using a mixture of  $\text{CHCl}_3$ -Me<sub>2</sub>CO (97:3), yielding 7 mg of chapinolin (2). The combined fractions 41-53

(78 mg), after further purification by prep TLC, furnished 12 mg of 3a and 8 mg of 4.

Chapinolin (2)  $\text{C}_{20}\text{H}_{26}\text{O}_5$ , gum, IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm<sup>-1</sup> 3400 (OH), 1760 ( $\gamma$ -lactone), 1765 (ester), 1635 (double bond); EIMS  $m/z$  (rel int) 346 [M]<sup>+</sup> (2), 246 [M-B]<sup>+</sup> (14), 228 [M-B-H<sub>2</sub>O]<sup>+</sup> (33), 213 [M-B-H<sub>2</sub>O-Me]<sup>+</sup> (19), 202 (25), 185 (10), 157 (15), 145 (13), 131 (16), 119 (16), 109 (19), 107 (16), 105 (20), 91 (24), 83 [B<sup>1</sup>]<sup>+</sup> (100), 55 [B<sup>2</sup>]<sup>+</sup> (49).

Skutchiolide A (3a)  $\text{C}_{22}\text{H}_{26}\text{O}_8$ , gum, IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm<sup>-1</sup> 3400 (OH), 1770 ( $\gamma$ -lactone), 1720 and 1710 (esters) 1630 (double bond), EIMS  $m/z$  (rel int) 418 [M]<sup>+</sup> (0.2), 358 [M-AcOH]<sup>+</sup> (2), 340 [M-AcOH-H<sub>2</sub>O]<sup>+</sup> (1), 302 [M-A]<sup>+</sup> (1), 284 [M-A-H<sub>2</sub>O]<sup>+</sup> (2), 242 [M-A-H<sub>2</sub>O-CH<sub>2</sub>CO]<sup>+</sup> (3), 225 (12), 214 (14), 199 (12), 185 (11), 169 (12), 147 (16), 116 [A]<sup>+</sup> (22), 83 [A<sup>1</sup>-O]<sup>+</sup> (14), 55 [A<sup>2</sup>-O]<sup>+</sup> (12), 43 [Ac]<sup>+</sup> (100).

Skutchiolide B (4)  $\text{C}_{22}\text{H}_{24}\text{O}_8$ , gum, IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm<sup>-1</sup> 1760 ( $\gamma$ -lactone), 1720 (ester), 1715 (ester), 1670, 1650, and 1620 (fragment B), EIMS  $m/z$  (rel int) 416 [M]<sup>+</sup> (2), 374 [M-CH<sub>2</sub>CO]<sup>+</sup> (1), 356 [M-AcOH]<sup>+</sup> (1), 317 [M-A<sup>1</sup>]<sup>+</sup> (0.4), 300 [M-A]<sup>+</sup> (5), 275 [M-CH<sub>2</sub>CO-A<sup>1</sup>]<sup>+</sup> (5), 258 [M-A-CH<sub>2</sub>CO]<sup>+</sup> (45), 241 [M-A-AcO]<sup>+</sup> (100), 229 (5), 213 (6), 185 (8), 161 (7), 135 (6), 116 [A]<sup>1</sup><sup>+</sup> (24), 43 [MeCO]<sup>+</sup> (76).

**Acknowledgements**—We thank Helga D Fischer for technical assistance. This research was supported, in part, by the US-Spain Joint Committee for Scientific and Technological Cooperation (Project No CCB-8409023).

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