3. Herz, W. and Watanabe, H. (1959) *J. Am. Chem. Soc.* **81**, 6088.
4. Herz, W., Watanabe, H., Miyazaki, M. and Kishida, Y. (1962) *J. Am. Chem. Soc.* **84**, 2601.
5. Rodriguez, E., Yoshioka, H. and Mabry, T. J. (1971) *Phytochemistry* **10**, 1145.
6. Sohi, A. S., Tiwari, V. D., Lonkar, A., Rangachar, S. K. and Nagasampagi, B. A. (1979) *Contact Dermatitis* **5**, 133.
7. Picman, A. K., Ranieri, R. L., Towers, G. H. N. and Lam, J. (1980) *J. Chromatogr.* **189**, 187.
8. Herz, W. and Hegnauer, G. (1961) *J. Org. Chem.* **26**, 5011.
9. Rodriguez, E. (1975) Ph. D. Thesis, University of Texas.
10. Mitchell, J. C., and Dupuis, G. (1971) *Br. J. Dermatol.* **84**, 139.
11. Subba Rao, P. V., Mangala, A., Towers, G. H. N. and Rodriguez, E. (1978) *Contact Dermatitis* **4**, 199.
12. Rodriguez, E., Dillon, M., Mabry, T. J., Mitchell, J. C. and Towers, G. H. N. (1976) *Experientia* **32**, 236.
13. Picman, A. K. and Towers, G. H. N. (in preparation).
14. Lonkar, A. and Jog, M. K. (1972) *Contact Dermatitis Newsl.* **7**, 291.
15. Rodriguez, E. (1975) *Phytochem Bull.* **8**, 7.
16. Yoshioka, H., Mabry, T. J. and Timmermann, B. N. (1973) *Sesquiterpene Lactones: Chemistry, NMR and Distribution.* University of Tokyo Press, Japan.

Phytochemistry, 1980, Vol. 19, pp. 2207 2209. © Pergamon Press Ltd. Printed in England. 0031 9422/80/1001-2207 $02.00/0

ACYCLIC DITERPENES FROM *CROTON KERRII*

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(*Revised received* 11 *January* 1980)

Key Word Index—*Croton kerrii*; Euphorbiaceae; acyclic diterpenes; anti-reserpine ulcer; (*E,E,Z*)-11-hydroxymethyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol; (*E,E,E*)-11-formyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol.

INTRODUCTION

Recently, we reported the structures of 18-hydroxygeranylgeraniol (**1**) [1], an anti-reserpine ulcer substance, and of plaunols A,B,C,D and E [2, 3], anti-Shay ulcer compounds, isolated from a Thai medicinal plant, *Croton sublyratus* Kurz. We have now commenced a chemotaxonomical survey of plants of the genus *Croton* which grow wild in Thailand. From *Croton* sp., various diterpenes belonging to the labdane (or clerodane) [4–10], pimarane [11], tiglane [12], crotofolane [13], and acyclic diterpene [1] have been isolated. We now report the isolation and characterization of two novel acyclic diterpenes from *Croton kerrii* A. Shaw (Euphorbiaceae) [14].

RESULTS AND DISCUSSION

A methanol extract of the leaves of *C. kerrii* was washed with *n*-hexane. The *n*-hexane extract was subjected to silica gel chromatography to yield diterpenes **2a** and **3a**. The diterpene diol (**2a**) had a molecular formula of $C_{20}H_{34}O_2$ by high resolution MS of its bis-trimethylsilylether (Calc. for $C_{26}H_{50}O_2Si_2$: 450, 3349. Found: 450, 3354). The IR, ^{1}H NMR, and MS of **2a** closely resembled those of **1**. On treatment with 3,5-dinitrobenzoyl chloride, **2a** gave a bis-3,5-dinitrobenzoate (**2c**), which confirmed that the two oxygen atoms were present as hydroxy groups. Thus **2a** possessed an acyclic tetraprenyl structure with two hydroxymethyl groups, one of which was placed on C-1, in view of its splitting pattern (doublet) in the ^{1}H NMR spectrum. **2a** was isomeric to **1** regarding the location of the extra hydroxyl group.

In order to determine the location of the hydroxyl group and the geometries of the double bonds in **2a**, detailed decoupling experiments were attempted by use of Eu(DPM)$_3$ as a shift reagent. By comparison with the ^{1}H NMR spectrum of **1**, all of the protons of **2a** were assigned as listed in Table 1. On irradiation of the signal at δ 5.21 (C-14) in the presence of the shift reagent, both broad singlets at 1.65 and 1.69 (C-16 and C-20) were sharpened showing that these two methyl groups and the olefinic proton were arranged on the same double bond and mutually underwent an allylic long-range coupling. Therefore, the hydroxyl group was situated neither on C-16 nor C-20. On the other hand, as irradiation of the signal at 6.44 (C-2) resulted in sharpening of the signal at 2.02 (C-17) and the collapsing of the doublet at 5.48 (C-1) to a singlet, the hydroxyl group could not be placed on C-17. Consequently, it must be located on C-19.

The geometry of the Δ^2-double bond was assigned as *E* by the $\Delta\delta$ values $\{\Delta\delta = \delta[\text{Eu(DPM)}_3] - \delta(CCl_4)\}$ (see Table 1) for the methyl (C-17) over the methylene groups (C-4) [1] and by the chemical shift of the former [15]. The chemical shift of the olefinic proton (C-10) and the shift

Table 1. ^{1}H NMR spectra of compounds **2a** and **2b**, and the effect of shift reagent*

	2a			**2b**		
	δ (CCl_4)	δ [Eu(DPM)$_3$]	$\Delta\delta$	δ (CCl_4)	δ [Eu(DPM)$_3$]	$\Delta\delta$
C-1	4.00 (*d*)	5.48† (7.53)‡	1.48 (3.53)	4.01 (*d*)	6.70§ (11.66)‖	2.69 (7.65)
C-2	5.30 (*t*)	6.44 (8.01)	1.14 (2.71)	5.30 (*t*)	7.63 (12.00)	2.33 (6.70)
C-4	2.04 (*m*)	2.55 (2.75)	0.31 (0.71)	2.05 (*m*)	2.65 (3.98)	0.60 (1.93)
C-5	2.04 (*m*)	2.34	0.30	2.05 (*m*)	2.63	0.58
C-6	5.05 (*t*)	5.48	0.43	5.06 (*t*)	5.86	0.80
C-8	2.04 (*m*)	2.33	0.29	2.05 (*m*)	2.75	0.70
C-9	2.04 (*m*)	2.88 (3.94)	0.84 (1.90)	2.05 (*m*)	2.75 (3.94)	0.70 (1.89)
C-10	5.18 (*t*)	5.64 (6.18)	0.46 (1.13)	5.30 (*t*)	7.35 (11.39)	2.05 (6.09)
C-12	2.04 (*m*)	2.51	0.47	2.05 (*m*)	2.95	0.90
C-13	2.04 (*m*)	2.34	0.30	2.05 (*m*)	2.60	0.55
C-14	5.05 (*t*)	5.21	0.16	5.07 (*t*)	5.40	0.33
C-16	1.65 (*s*)	1.69	0.04	1.66 (*s*)	1.74	0.08
C-17	1.63 (*s*)	2.02 (2.54)	0.39 (0.91)	1.63 (*s*)	2.36 (3.72)	0.73 (2.14)
C-18	1.58 (*s*)	1.82	0.24	1.58 (*s*)	2.02	0.44
C-19	3.98 (*s*)	5.31 (7.27)	1.33 (3.29)	3.89 (*s*)	6.46 (11.64)	2.57 (7.75)
C-20	1.58 (*s*)	1.65	0.07	1.58 (*s*)	1.74	0.06

* Recorded at 100 MHz in CCl_4. Chemical shifts are in ppm with Me_4Si as an internal standard.
† Measured in CCl_4 containing 20% Eu(DPM)$_3$.
‡ 50%.
§ 40%.
‖ 50%.

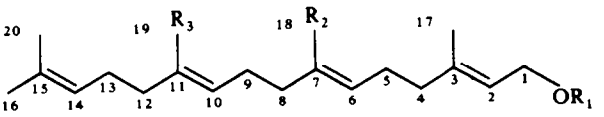
effect indicated the Δ^{10}-double bond to be *Z* [1, 16]. In the same manner, the Δ^6-bond may be assigned as *E* from the chemical shift of the methyl group (C-18) [1, 15]. Consequently, **2a** must be depicted as [*E,E,Z*]-11-hydroxymethyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol.

The diterpene aldehyde (**3a**) was purified by acetylation. The IR (ν_{max}^{liquid} cm^{-1}: 1740, 1690), UV [λ_{max}^{EtOH} nm (log ε): 230 (4.0)], and ^{1}H NMR spectra (δ 9.26, C-19) of its acetate (**3b**) showed the presence of an α,β-unsaturated aldehyde, whose conjugated double bond can be assigned as *E* based on the high-field chemical shift of its aldehydic proton compared with those of the model compounds, (*E*)- and (*Z*)-2-methyl-2-pentenal [16]. Reduction of **3b** with $LiAlH_4$ gave a diol (**2b**). In the ^{1}H NMR spectrum of **2b** (Table 1), the $\Delta\delta$ values for the olefinic proton (C-10) over the methylene group (C-9) agreed with the above assignment. The other structural features of **2b** were proved to be the same as those of **2a** by the spectral data. Therefore, **3a** should be shown as (*E,E,E*)-11-formyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol.

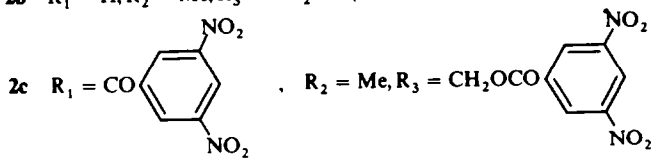
Finally, **2a** was proved to be slightly less active than **1** and to be equally active to its isomer (**2b**) in an inhibitory assay against reserpine-induced ulcer in mice.

EXPERIMENTAL

Extraction and isolation. Powdered leaves (1.5 kg) of *C. kerrii* were extracted 3 × with MeOH under reflux. After evapn of the solvent, the residue was refluxed in *n*-hexane with vigorous stirring. The *n*-hexane layer was concd to dryness to yield 31 g of a dark green oil, 10 g of which was subjected to Si gel chromatography. Elution with C_6H_6–EtOAc (5:1) gave 332 mg of crude **3a**. Elution with C_6H_6–EtOAc (2:1) afforded 612 mg of **2a** as a colourless oil. **2a**: IR ν_{max}^{liquid} cm^{-1}: 3340, 1665, 1445, 1380, 1000. MS (probe) 75 eV, *m/e* (rel. int.): 288 (< 1), 273 (< 1), 270 (< 1), 121 (22), 81 (52), 69 (100). (Found: C, 78.20; H, 11.06. $C_{20}H_{34}O_2$ requires: C, 78.43; H, 11.11%).



1 $R_1 = H, R_2 = CH_2OH, R_3 = Me$
2a $R_1 = H, R_2 = Me, R_3 = CH_2OH$
2b $R_1 = H, R_2 = Me, R_3 = CH_2OH$ (Δ^{10}: *E*)



2c $R_1 = CO$, $R_2 = Me, R_3 = CH_2OCO$

3a $R_1 = H, R_2 = Me, R_3 = CHO$ (Δ^{10}: *E*)
3b $R_1 = Ac, R_2 = Me, R_3 = CHO$ (Δ^{10}: *E*)

Acetylation of **3a**. To a soln of 332 mg **3a** in 5 ml CH_2Cl_2 was added 0.5 ml Ac_2O and 0.5 ml pyridine. After usual work-up, the residue was purified by PLC developed 3 × with C_6H_6–EtOAc (10:1) to yield 43 mg **3b** as a colourless oil. IR ν_{max}^{liquid} cm^{-1}: 1740, 1690, 1640, 1240; ^1H NMR (100 MHz, $CDCl_3$): δ 1.57 (3 H, *s*), 1.64 (3 H, *s*), 1.66 (3 H, *s*), 1.70 (3 H, *s*), 1.96 (3 H, *s*), 1.9–2.4 (12 H, *m*), 4.47 (2 H, *d*, J = 7.0 Hz), 4.8–5.3 (3 H, *m*), 6.28 (1 H, *t*, J = 7.0 Hz), 9.26 (1 H, *s*). UV λ_{max}^{EtOH} nm: (log ε): 230 (4.0). (Found: C, 76.02; H, 10.04. $C_{22}H_{34}O_3$ requires: C, 76.30; H, 9.83%).

Esterification of **2a** *with 3,5-dinitrobenzoyl chloride*. To a soln of 150 mg **2a** in 3 ml CH_2Cl_2 was added 250 mg 3,5-dinitrobenzoyl chloride and 0.5 ml pyridine. After usual work-up, the residue was purified by PLC developed with C_6H_6-EtOAc (3:1) to yield 313 mg **2c** as a colourless oil. IR ν_{max}^{liquid} cm^{-1}: 1740, 1735, 1630, 1600, 1560. ^1H NMR (60 MHz, $CDCl_3$): δ 1.54 (3 H, *s*), 1.59 (6 H, *s*), 1.77 (3 H, *s*,), 1.9 2.3 (12 H, *m*), 4.8–5.3 (6 H, *m*), 5.54 (2 H, *d*, J = 7.0 Hz), 9.22 (6 H, *s*). (Found: C, 58.62; H, 5.42; N, 8.18. $C_{34}H_{38}N_4O_{12}$ requires: C, 58.84; H, 5.52; N, 8.06%).

$LiAlH_4$ reduction of **3b**. To a soln of 10 mg $LiAlH_4$ in 2 ml dry Et_2O was added a soln of 30 mg **3b** in 1 ml Et_2O at 0°. After usual work-up, the residue was subjected to Si gel chromatography. Elution with C_6H_6–EtOAc (2:1) gave 25 mg of **2b** as a colourless oil. IR ν_{max}^{liquid} cm^{-1}: 3340, 1665, 1445, 1380, 1000. MS (probe) 75 eV, *m/e* (rel. int.): 288 (< 1), 273 (< 1), 270 (< 1), 121 (29), 81 (67), 69 (100). (Found: C, 78.61; H, 10.95. $C_{20}H_{34}O_2$ requires: C, 78.43; H, 11.11%).

Acknowledgements – We are indebted to Mr. Ananta Laophanich of Asian TJD Enterprize Ltd., Thailand and Mr. Chana Promdej of Royal Forest Department in Thailand for their collection of the original plant.

REFERENCES

1. Ogiso. A., Kitazawa, E., Kurabayashi, M., Sato, A., Takahashi, S., Noguchi, H., Kuwano, H., Kobayashi, S. and Mishima, H. (1978) *Chem. Pharm. Bull. Jpn* **26**, 3117.
2. Kitazawa, E., Ogiso, A., Sato, A., Kurabayashi, M., Kuwano, H., Hata, T. and Tamura, C. (1979) *Tetrahedron Letters* 1117.
3. Kitazawa, E., Sato, A., Takahashi, S., Kuwano, H. and Ogiso, A. (1980) *Chem. Pharm. Bull. Jpn* **28**, 227.
4. Rojas, E. T. and Rodriquez-Hohn, L. (1978) *Phytochemistry* **17**, 574.
5. Chatterjee, A., Banerjee, A. and Bohlman, F. (1977) *Tetrahedron* **33**, 2407.
6. Chatterjee, A., Banerjee, A. and Bohlman, F. (1978) *Phytochemistry* **17**, 1777.
7. Aiyar, V. N. and Seshadri, T. R. (1972) *Phytochemistry* **11**, 1473.
8. Bruke, B. A., Chan, W. R., Prince, E. C., Eickman, N. and Clardy, J. (1976) *Tetrahedron* **32**, 1881.
9. de Alvarega, M. A., Gottleib, H. E., Gottleib, O. R., Magalhaes, M. T. and da Silva, V. O. (1978) *Phytochemistry* **17**, 1773.
10. Birtwistle, J. S., Case, D. E., Dutta, P. S., Holsal, T. G., Mattewo, G., Sabel, H. D. and Thaller, V. (1962) *Proc. Chem. Soc.* 329.
11. Aiyar, V. N. and Seshadri, T. R. (1972) *Curr. Sci.* **41**, 839.
12. Hecker, E. and Schmidt, R. (1974) *Fortschr. Chem. Org. Naturst.* **31**, 377.
13. Chan, W. R., Prince, E. C., Manchand, P. S., Springer, J. P. and Clardy, J. (1975) *J. Am. Chem. Soc.* **97**, 4437.
14. Shaw, A. (1972) *Kew Bull.* **26**, 191.
15. Bates, R. B. and Gale, D. M. (1960) *J. Am. Chem. Soc.* **82**, 5749.
16. Chan, K. C., Jewell, R. A., Nutting, W. H. and Rapoport, H. (1968) *J. Org. Chem.* **33**, 3382.