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(−)-ALLO-PERTUSARIC ACID AND (−)-DIHYDROPERTUSARIC ACID FROM THE LICHEN *PERTUSARIA ALBESCENS**

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Key Word Index—*Pertusaria albescens*; *P. ophthalmiza*; Pertusariaceae; lichens; (−)-allo-pertusaric acid; (−)-dihydropertusaric acid; γ-lactones; taraxerone.

Abstract—The structures of two γ-lactone carboxylic acids from the lichen *Pertusaria albescens*, (−)-allo-pertusaric acid and (−)-dihydropertusaric acid, have been elucidated by spectroscopic and chemical methods. From *P. ophthalmiza*, taraxerone and a mixture of long chain aliphatic alcohols and fatty acids have been isolated.

INTRODUCTION

In 1898 Hesse [1] isolated from *Pertusaria communis* D.C. *β-variolosa* Wallr. (= *P. albescens*) the lichen substances pertusaric acid, mp 103°, $C_{23}H_{36}O_6$ or $C_{24}H_{38}O_6$, pertusarin, mp 235°, $C_{30}H_{50}O_2$, pertusaren and pertusaridin. Three years later the same author [2] described from the same species a compound of mp 82°, $C_{22}H_{36}O_7$, which he named orbiculatic acid. Recently Hanko [3] analysed numerous specimens of *P. albescens* (Huds.) Choisy et Werner by TLC and mass spectrometry and found the fatty acids 'bH' and 'bH1' as major and minor components. According to Hanko [3] the peaks at *m/z* 366 and *m/z* 368 in the lichen mass spectrum correspond to the $[M]^+$ peaks of the compounds 'bH' and 'bH1', respectively. The remission UV spectrum of 'bH' had a maximum at 232 nm, identical with that of lichesterinic acid. By reason of these findings Hanko proposed γ-lactonic structures for 'bH' and 'bH1'.

To find the true identity of compounds 'bH' and 'bH1' we reinvestigated *P. albescens* and we describe the isolation and structural elucidation of the new γ-lactone carboxylic acids (−)-allo-pertusaric acid and (−)-dihydropertusaric acid in this paper.

RESULTS AND DISCUSSION

The ether extract of *P. albescens* showed on silica gel TLC two spots with R_f 0.31 and 0.36 (*n*-hexane–Et_2O–HCO_2H–30:20:6) obviously corresponding to (−)-allo-pertusaric acid (**1**) and (−)-dihydropertusaric acid (**2**), respectively. Prep. TLC of the mixture with the same adsorbent and solvent mixture gave two bands of the R_f values 0.35 and 0.42, which were extracted with ether. The lower band yielded a product of λ_{max}^{MeOH} 211 nm (log ε4.0), identical with allo-pertusaric acid. The product from the upper band showed a broad UV absorption with a maximum at 228 nm (log ε3.80) and a shoulder at 210 nm (log ε3.67) which proved the presence of a mixture of **2** and acid **3**; hence isomerization of **1** to **3** had occurred during prep. TLC. This may be the reason for Hanko's statement [3] that the UV spectra of his compound '1H' and lichesterinic acid are identical. Experiments to separate **1** and **2** by fractional crystallization from different solvents were unsuccessful. However, it was found that the crystalline sodium salt of **1** is insoluble in a cold solution of sodium hydrogen carbonate and can be separated from the easily soluble sodium salt of **2** by filtration. By this method separation of **1** and **2** on a preparative scale was achieved. (−)-Allo-pertusaric acid crystallized from methanol in lustrous plates of mp 74–76° and $[\alpha]_D^{24}$ −95.5, had according to the high resolution mass spectrometry the formula $C_{21}H_{34}O_5$ and showed in its UV spectrum (in methanol) a maximum at 209 nm (log ε3.92), indicative of an α-methylene-γ-lactone. The ^1H NMR spectrum (Table 1) of (−)-allo-pertusaric acid gave the structure and relative stereochemistry shown in formula **1**. Inspection of a model shows that in the most stable conformation of the molecule the protons at C-3 and C-4 include an angle of *ca* 15° which corresponds according to the Karplus relation to a coupling constant of *ca* 9 Hz, in good agreement with the value of 8 Hz observed. Furthermore, the NMR data are in excellent agreement with the corresponding data of (−)-allo-protolichesterinic acid [4]. The absolute configuration of (−)-allo-pertusaric acid as shown in formula **1** follows from the nearly identical CD and ORD spectra (Fig. 1) of **1** and (−)-allo-protolichesterinic acid.

(−)-Allo-pertusaric acid gave on heating with acetic anhydride a compound of mp 96–98°, $[\alpha]_D^{23}$ −30* and λ_{max}^{MeOH} 229 nm (log ε4.22), identical with (−)-isomuronic acid (**3**) (= (−)-dehydroconstipatic acid) [5]. The ORD

*Part 144 in the series "Lichen Substances". For Part 143 see Huneck, S. and Tibell, L., *J. Hattori Bot. Lab.* (in press).

*$[\alpha]_D^{25}$ −73.5 ($CHCl_3$) in ref. [5] is a misprint according to a letter of Dr. J. A. Elix of 4 April 1984. The correct value is $[\alpha]_D^{25}$ −23.5 ($CHCl_3$). We are grateful to Dr. Elix for this information.

Table 1. ^{1}H NMR data of compounds **1–9**, **11**, **14**, **19** and **20** (400 MHz, $CDCl_3$, J in Hz)

Compound No.	2-H	3-H	4-H	17-H_2	19-H_3	20-H_3	Other protons
1	—	4.02, *ddd* $J_{3,4}=8.0$ $J_{3,20}=2.0$	4.69, *ddd* $J_{4,3}=8.0$ $J_{4,5}=8.0, 11.5$	2.43, *t* $J=8.0$	2.15, *s*	—	20-H_A: 6.45, *d*, $J=2.0$ 20-H_B: 5.89, *d*, $J=2.0$
2	3.02, *dq* $J_{2,3}=10.0$ $J_{2,20}=7.2$	3.20, *dd* $J_{3,2}=10.0$ $J_{3,4}=8.0$	4.67, *ddd* $J_{4,3}=8.0$ $J_{4,5}=5.0, 8.5$	2.41, *t* $J=7.5$	2.13, *s*	1.26, *d* $J_{20,2}=7.2$	—
3	—	—	5.12, *ddq* $J_{4,5}=3.0, 9.0$	2.43, *t* $J=7.5$	2.15, *s*	2.23, *d* $J_{20,4}=2.0$	—
5	—	3.41, *d* $J_{3,4}=6.0$	5.31, *ddd* $J_{4,3}=6.0$ $J_{4,5}=5.0, 9.0$	2.40, *t* $J=7.5$	2.11, *s*	—	21-H_3: 3.75, *s* 20-H_2: 1.80, *m* 22-H_2: 4.80, *m*
6	2.92, *dq* $J_{2,3}=7.5$ $J_{20,2}=7.0$	3.31, *dd* $J_{3,2}=7.5$ $J_{3,4}=5.5$	4.42, *ddd* $J_{4,3}=5.5$ $J_{4,5}=5.0$	2.41, *t* $J=7.5$	2.13, *s*	1.28, *d* $J_{20,2}=7.0$	
7	2.88, *dq* $J_{2,3}=7.5$ $J_{2,20}=7.0$	3.31, *dd* $J_{3,2}=7.5$ $J_{3,4}=5.5$	4.39, *ddd* $J_{4,3}=5.5$ $J_{4,5}=5.0$	2.40, *t* $J=7.5$	2.12, *s*	1.20, *d* $J_{20,2}=7.5$	21-H_3: 3.73, *s*
8	3.02, *dq* $J_{2,3}=10.0$ $J_{2,20}=7.5$	3.15, *dd* $J_{3,2}=10.0$ $J_{3,4}=8.0$	4.62, *ddd* $J_{4,3}=8.0$ $J_{4,5}=3.0, 10.0$	2.39, *t* $J=7.5$	2.11, *s*	1.25, *d* $J_{20,2}=7.5$	21-H_3: 3.74, *s*
9	2.95, *dq* $J_{2,3}=10.0$ $J_{2,20}=7.0$	2.64, *dd* $J_{3,2}=9.5$ $J_{3,4}=11.5$	4.44, *ddd* $J_{4,3}=9.5$ $J_{4,5}=4.5, 8.0$	2.41, *t* $J=7.5$	2.12, *s*	1.32, *d* $J_{20,2}=7.5$	21-H_3: 3.77, *s*
11	3.03, *dq* $J_{2,3}=9.5$ $J_{2,20}=7.0$	3.22, *dd* $J_{3,2}=10.0$ $J_{3,4}=8.5$	4.70, *ddd* $J_{4,3}=8.5$ $J_{4,5}=4.0, 9.0$		1.75, *s*	1.33, *d* $J_{20,2}=7.5$	–S–CH_2–CH_2–S–: 3.32, *m*
14	3.05, *dq* $J_{2,3}=10.0$ $J_{2,20}=7.0$	3.17, *dd* $J_{3,2}=10.0$ $J_{3,4}=8.0$	4.64, *ddd* $J_{4,3}=8.0$ $J_{4,5}=3.0, 10.0$		0.88, *t* $J=7.5$	1.30, *d* $J_{20,2}=7.0$	21-H_3: 3.76, *s*
19	—	3.03, *d* $J_{3,4}=6.5$	4.67, *ddd* $J_{4,3}=6.5$ $J_{4,5}=5.0, 9.5$	2.41, *t* $J=7.5$	2.12, *s*	—	21-H_3: 3.73, *s*, 20-H_2, 22-H_2 0.98, *m*; 1.09, *m*
20	—	3.84, *ddd* $J_{3,4}=7.0$ $J_{3,20}=2.0$ $J_{3,22}=1.5$	4.50, *ddd* $J_{4,3}=7.0$ $J_{4,5}=5.0, 10.0$	2.41, *t* $J=7.5$	2.12, *s*	—	21-H_3: 3.72, *s* 20-H_2: 6.41, *dq*, $J_{20,22}=7.5$, $J_{22,3}=1.5$ 22-H_3: 2.21, *dd*, $J_{22,20}=7.5$; $J_{3,22}=1.5$

spectrum (in methanol) of **3**

$$\left([\alpha]^{24}\ \begin{matrix}-50 & -100 & 0 & +225\\ 350 & 286\ 270 & & 260\,\text{nm}\end{matrix}\right)$$

was in good agreement with the ORD spectrum (in methanol) of (−)-lichesterinic acid

$$\left([\alpha]^{24}\ \begin{matrix}-100 & -152 & 0 & +254\\ 350 & 286\ 270 & & 260\,\text{nm}\end{matrix}\right)$$

and confirmed again the *S*-configuration at C-4 of **3**. The reason for this isomerization is the *cis* position of all substituents in the molecule. Acid **3** gave with diazomethane methyl (−)-*iso*-muronate (**4**). Addition of diazomethane to **1** yielded the methyl ester pyrazoline **5** with two negative CD bands at 325 ($\Delta\varepsilon$ −6.8) and 208 nm ($\Delta\varepsilon$ −1.4) which corresponds according to Snatzke's rule of the –N=N–C–CO– chromophore [6] to the stereochemistry shown in **5**, resulting from a si attack of diazomethane on **1**. (−)-Allo-pertusaric acid gave on hydrogenation with Pd–C in acetic acid followed by reoxidation with Jones' reagent (−)-dihydro-allo-pertusaric acid (**6**), which yielded the corresponding methyl ester **7** with diazomethane.

(−)-Dihydropertusaric acid crystallized in small needles of mp 105–107°, $[\alpha]_D^{24}$ −74.9 and had according to the

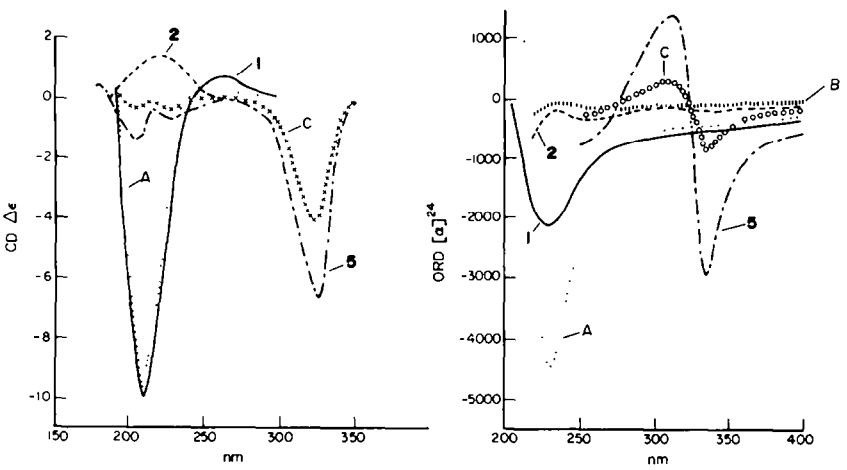


Fig. 1. CD and ORD spectra of compounds **1**, **2**, **5**, (−)-allo-protolichesterinic acid (A), (−)-dihydroprotolichesterinic acid (B) and (−)-allo-protolichesterinic acid methyl ester pyrazoline (C).

high resolution mass spectrum the formula $C_{21}H_{36}O_5$; it showed in its UV spectrum (in methanol) a maximum at 207 nm (log ε2.74), indicative of a saturated γ-lactone. The ^{1}H NMR spectrum (Table 1) and ORD data of (−)-dihydropertusaric acid gave the structure and absolute configuration shown in formula **2**. The methyl ester of **2** (**8**) and **7** were isomerized with sodium methylate to methyl (−)-neo-dihydropertusarate (**9**), the most stable isomer in this series. The remaining fourth isomer, pertusarinic acid (**10**) is unknown at the present time.

The ORD curve of methyl (−)-dihydropertusarate (**8**) is very similar to the ORD curve of methyl (−)-dihydro-protolichesterinate (Fig. 2), thus proving the same relative and absolute configuration of both compounds. Contrary to this the ORD spectra of methyl (−)-dihydro-allo-pertusarate (**7**) and methyl (−)-neodihydropertusarate (**9**) were quite different (Fig. 2). As Table 1 shows, the signal of H-4 in **8** (δ4.67) is shifted downfield in relation to the chemical shift of H-4 in **7** (δ4.42). This shift is caused by the deshielding of the H-4 through the $-CO_2R$ carbonyl group in **8** and establishes again the 3α-position of the $-CO_2R$ group in **2** and **8**. Hence (−)-dihydropertusaric acid is 2(*S*)-methyl-3(*R*)-carboxy-4(*S*)-hydroxy-18-oxononadecan-1 → 4-olide (**2**).

(−)-Dihydropertusaric acid reacted with ethanedithiol to yield the dithiospiroketal **11** which gave with diazomethane the oily methyl ester **12**. Desulphuration of **11** with Raney-Ni yielded (−)-pertusaranic acid (**13**), a homologue of (−)-dihydroprotolichesterinic acid. Methylation of **13** with diazomethane gave the methyl ester **14**. The ORD spectra of **11**, **13** and **14** are similar to the ORD spectrum of **2**, thus proving the same stereochemistry of **2** and **11**–**14**. Dertien *et al.* [7] isolated from *Cladonia impexa* Harm. the methyl ester of a saturated γ-lactonic acid, $C_{22}H_{40}O_4$, but unfortunately the physical data of this compound were not given. Acids **2** and **3** gave with hydroxylamine in pyridine the corresponding oximes **15** and **16**.

On isomerization of **8** with sodium methoxide a minor product was observed which had according to the ^{1}H NMR spectrum (400 MHz, $CDCl_3$) structure **17**: δ1.32 (*d*, *J* = 7.5 Hz, 3H, 20-H_3), 2.13 (*s*, 3H, 19-H_3), 2.41 (*t*, *J* = 7.5 Hz, 2H, 17-H_2), 2.47 (*q*, *J* = 7.5 Hz, 2H, 5-H_2),

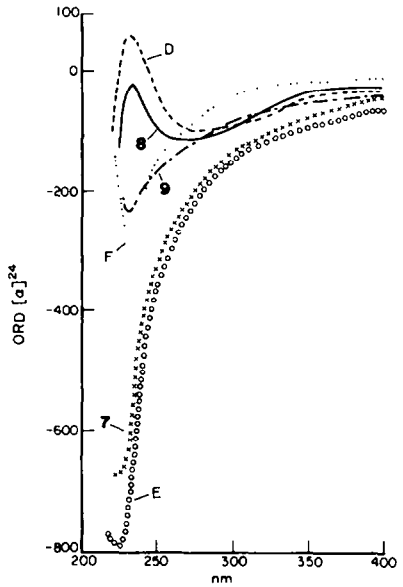
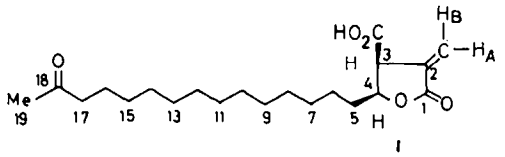


Fig. 2. ORD spectra of compounds **7**, **8**, **9**, methyl-(−)-dihydro-protolichesterinate (D), methyl (−)-dihydro-allo-protolichesterinate (E) and methyl (−)-neo-dihydro-protolichesterinate (F).

3.47 (*q*, *J* = 7 Hz, 1H, 2-H), 3.67 (*s*, 3H, 1-CO_2Me), 3.73 (*s*, 3H, 21-CO_2Me), 6.03 (*t*, *J* = 7.5 Hz, 1H, 4-H).

Reduction of **8** with sodium borohydride gave the rearranged lactone **18** with the following ^{1}H NMR data (400 MHz, $CDCl_3$): δ1.08 (*d*, *J* = 7 Hz, 3H, 20-H_3), 1.17 (*d*, *J* = 6 Hz, 3H, 19-H_3), 2.53 (*t*, $J_{3,2} = J_{3,4} = 8$ Hz, 1H, 3-H), 2.64 (*dq*, $J_{2,3} = 1.5$ Hz, $J_{2,20} = 7$ Hz, 1H, 2-H), 3.88 (*dt*, $J_{4,3} = 8$ Hz, $J_{4,5} = 2.5$ Hz, 1H, 4-H), 3.78 (*m*, 1H, 18-H), 4.28 (*dd*, *J* = 5.5 and 9 Hz, 2H, 1-H_2). A strong carbonyl band at 1770 cm^{-1} in the IR spectrum of **18** (in carbon tetrachloride) confirmed the presence of a γ-lactone. The methyl ester pyrazoline **5** gave on reaction with conc. H_2SO_4 the cyclopropyl compound **19**, whilst



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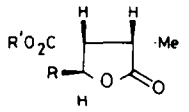


2–10 R = Me-CO-$(CH_2)_{13}$-

2 R' = H

8 R' = Me

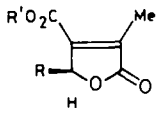
11 R = Me–C(S–S)–$(CH_2)_{13}$-, R' = H

12 R = Me–C(S–S)–$(CH_2)_{13}$-, R' = Me

13 R = n-$C_{15}H_{31}$-, R' = H

14 R = n-$C_{15}H_{31}$-, R' = Me

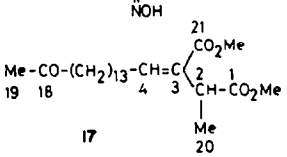
15 R = Me-C(=NOH)-$(CH_2)_{13}$-, R' = H



3 R' = H

4 R' = Me

16 R = Me-C(=NOH)-(CH_{13})-, R' = H



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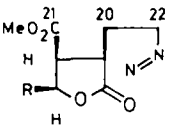

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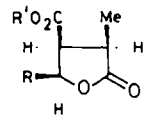
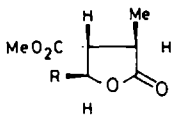


6 R' = H

7 R' = Me



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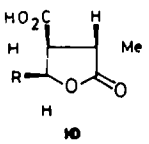

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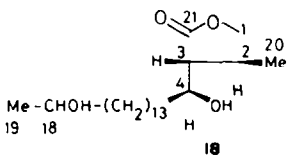

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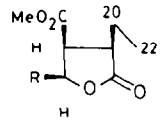
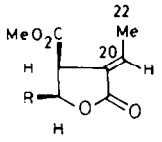


19 R = Me-CO-$(CH_2)_{13}$-



20 R = Me-CO-$(CH_2)_{13}$-

thermolysis of **5** yielded the homo-compound **20**.

Sodium (−)-allo-pertusarate and potassium (−)-dihydropertusarate showed in the concentration range 10^{-2}–10^{-4} M growth inhibitory properties on seedlings of *Lepidium sativum*, *Triticum aestivum* and *Pisum sativum* (Table 2). Furthermore (−)-allo-pertusaric acid irritates the mucous membranes in the nose and pharynx and causes sneezing and coughing.

The ether extract of *Pertusaria ophthalmiza* (Nyl.) Nyl. from Norway showed in accordance with the findings of Hanko [3] the presence of numerous components by TLC. One main component could be separated by CC and crystallization and proved to be identical with taraxerone. This is the first detection of taraxerone in a lichen. Hesse's pertusarin [1] is very probably identical with this triterpene. The following other components of *P. ophthalmiza* were identified by mass spectrometry: $C_{26}H_{51}OH$, $C_{28}H_{55}OH$, $C_{30}H_{59}OH$, $C_{21}H_{43}CO_2H$ (behenic acid), $C_{23}H_{47}CO_2H$ (lignocerinic acid), $C_{25}H_{51}CO_2H$ (cerotic acid), $C_{27}H_{55}CO_2H$ (octacosanoic acid) and $C_{29}H_{59}CO_2H$ (melissic acid). However, it should be mentioned that the bark of *Alnus glutinosa* and *A. incana* on which *P. ophthalmiza* often grows, contain taraxerone and taraxerol, respectively [8]. A final decision whether taraxerone and the long chain fatty acids mentioned are genuine constituents of the lichen or not could not be made because it is practically impossible to separate the crustose thallus of the lichen from the substrate.

EXPERIMENTAL

MS were recorded at the 'Manfred von Ardenne' Research Institute Dresden, East Germany, with an unheated ion source, inlet temp. 90–120°, electron current 10 mA and an accelerating voltage of 40 kV.

Analysis of P. albescens. Air dried and ground lichen (357 g, Norway, Hordaland, Bergen, 60 m a.s., on bark of deciduous trees, leg. et det. T. Tønsberg, 9.7.1984 coll. no. 8863; voucher specimen in the Herbarium of the Botanical Institute of the University of Bergen (BG), Norway) was extracted with Et_2O (500 ml) for 12 hr and the extract shaken with an ice-cold satd soln of $NaHCO_3$ in H_2O for 15 min. The lustrous crystals of Na (−)-allo-pertusarate which were separated at the interphase were removed by filtration, washed with a few ml of $NaHCO_3$ soln and converted into the acid **1** by shaking with excess H_2SO_4 (2%) and Et_2O. The Et_2O soln was washed with H_2O, dried (Na_2SO_4) and then taken to dryness. The residue was recrystallized twice from MeOH–H_2O and yielded (−)-allo-pertusaric acid (**1**) (6 g, 1.68%) in lustrous plates mp 76–78° and

$[\alpha]^{24}$	−95.5	−101.6	−122.6	−207.2	−253.7	−340.0
	589	578	546	436	406	340 nm

(MeOH; *c* 1.525). R_f 0.23 (silica gel PF 254+366, toluene–HOAc, 20:3). IR ν_{max}^{KBr} cm^{-1}: 728, 830, 874, 998, 1010, 1026, 1184, 1260, 1342, 1410, 1470, 1658, 1700, 1738, 2950, 3150.

The mother liquor of Na (−)-allo-pertusarate was acidified

Table 2. Growth regulating properties of sodium (−)-allo-pertusarate and potassium (−)-dihydropertusarate.

Concentration: (mol/l)	Length of seedling (% of control)														
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	Lepidium sativum					*Triticum aestivum*					*Pisum sativum*				
Sodium (−)-allo-pertusarate	0	3	44	98	—	0	11	107	118	—	—	—	—	—	—
Potassium (−)-dihydropertusarate	0	0	80	85	106	14	23	37	53	68	94	65	105	100	125
Sodium (−)-allo pertusarate	1	1	71	100	—	0	0	92	86	—	4	114	90	100	—
Potassium (−)-dihydropertusarate	0	0	110	98	105	0	6	46	42	77	29	100	120	100	113

with H_2SO_4 (5%) and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried (Na_2SO_4) and the residue after evapn of solvent recrystallized ×3 from MeOH–H_2O: (−)-*dihydropertusaric acid* (**2**) (5 g, 1.4%) in small needles mp 105–107° and

$$[\alpha]^{24}\ \frac{-74.9\ \ -90.2\ \ -148.8\ \ -175.3\ \ -223.2}{589\quad 578\quad 436\quad 406\quad 366\ \text{nm}}$$

(MeOH; *c* 2.15). R_f 0.27 (silica gel PF 254 + 366, toluene–HOAc, 20:3), R_f 0.59 (silica gel PF 254 + 366, C_6H_6–dioxane–HOAc, 36:10:1.6). IR ν_{max}^{KBr} cm^{-1}: 702, 726, 860, 878, 988, 1014, 1090, 1112, 1194, 1198, 1242, 1318, 1366, 1418, 1470, 1736, 2970, 3180. ^{13}C NMR ($CDCl_3$): C-1: 173.04 (*s*), C-2: 36.65 (*d*), C-3: 51.73 (*d*), C-4: 77.47 (*d*), C-5: 31.17 (*t*), C-6: 25.58 (*t*), C-7–C-15: 29.14–29.43 (9 × *t*), C-16: 23.95 (*t*), C-17: 43.80 (*t*), C-18: 209.82 (*s*), C-19: 29.54 (*q*), C-20: 14.41 (*q*), C-21: 177.33 (*s*). MS *m/z* (rel. int.): 368 $[M]^+$ (16%), 353 (6), 339 (4), 326 (10), 325 (12), 293 (34), 292 (12), 275 (38), 265 (18), 247 (16), 219 (12), 191 (16), 179 (32), 178 (22), 137 (18), 123 (22), 109 (36), 97 (38), 95 (58), 85 (20), 83 (38), 81 (56), 71 (56), 71 (56), 69 (68), 67 (42), 59 (22), 58 (82), 55 (100).

$$\text{CD, } \Delta\varepsilon\ \frac{+0.058\ \ +0.104\ \ +1.38\ \ +0.125}{280\quad 260\quad 221\quad 192\ \text{nm}}\ \text{(MeCN)}.$$

(−)-*Iso-muronic acid* (**3**). Prepared by heating **1** (0.2 g) with Ac_2O (5 ml) at 100° for 1 hr. After usual work up, prep. TLC and crystallization from MeOH flat needles mp 96–98° and

$$[\alpha]^{24}\ \frac{-30.0\ \ -31.7\ \ -37.6\ \ -57.6\ \ -67.0\ \ -76.4}{589\quad 578\quad 546\quad 436\quad 406\quad 366\ \text{nm}}$$

(MeOH; *c* 0.85). $C_{21}H_{34}O_5$ (366.48). IR ν_{max}^{KBr} cm^{-1}: 724, 766, 890, 964, 992, 1010, 1030, 1160, 1220, 1290, 1320, 1350, 1370, 1430, 1464, 1702, 1730, 2980, 3200. MS *m/z* (rel. int.): 366 ($[M]^+$, 34%), 349 (14), 348 (46), 320 (14), 309 (22), 308 (12), 291 (12), 290 (20), 263 (26), 253 (30), 251 (20), 239 (32), 225 (36), 211 (36), 197 (18), 179 (20), 155 (80), 142 (16), 125 (16), 123 (26), 121 (16), 111 (26), 109 (32), 97 (48), 95 (52), 85 (32), 83 (60), 81 (52), 79 (18), 71 (78), 69 (100), 67 (56).

Me (−)-*iso-muronate* (**4**). Prepared from **3** with CH_2N_2 in Et_2O at 20° for 5 min. Needles mp 58–59° (from MeOH) and

$$[\alpha]^{24}\ \frac{-27.6\ \ -27.6\ \ -27.6\ \ -51.3\ \ -51.3\ \ -43.4}{589\quad 578\quad 546\quad 436\quad 406\quad 366\ \text{nm}}$$

($CHCl_3$; *c* 0.253). $C_{22}H_{36}O_5$ (380.51). IR ν_{max}^{KBr} cm^{-1}: 730, 764, 800, 960, 1016, 1054, 1084, 1106, 1162, 1234, 1304, 1346, 1434, 1472, 1722 (–CO_2Me, –COMe), 1760 (γ-lactone CO), 2980. UV λ_{max}^{MeOH} (log ε): 230 nm (4.14). MS *m/z* (rel. int.): 380 ($[M]^+$, 62%), 365 (11), 348 (62), 323 (84), 307 (44), 293 (53), 275 (61), 267 (51), 253 (61), 239 (51), 191 (47), 179 (64), 169 (100), 146 (87), 135 (63), 129 (84), 128 (70), 127 (58), 123 (72), 121 (66), 109 (85), 101 (59), 97 (74), 95 (92).

Addition product of diazomethane with (−)-*allo-pertusaric acid* (**5**). Prepared from **1** (0.5 g) with CH_2N_2 in Et_2O at 20° for 3 hr. Prismatic plates mp 63–65° (from MeOH) and

$$[\alpha]^{24}\ \frac{-163.6\ \ -173.0\ \ -204.5\ \ -393.5\ \ -523.0\ \ -930.5}{589\quad 578\quad 546\quad 436\quad 406\quad 366\ \text{nm}}$$

(MeOH; *c* 2.00). $C_{23}H_{38}N_2O_5$ (422.55). IR ν_{max}^{KBr} cm^{-1}: 732, 904, 1010, 1099, 1170, 1214, 1254, 1290, 1310, 1372, 1444, 1470, 1732 (–CO_2Me, –CO–Me), 1778 (γ-lactone CO), 3000. MS *m/z* (rel. int.): 394 $[M-N_2]^+$.

(−)-*Dihydro-allo-pertusaric acid* (**6**). Prepared to hydrogenation of **1** (0.4 g) in HOAc (20 ml) with Pd–C (10%, 0.4 g) under normal conditions for 1 hr. The catalyst was removed by filtration, the solvent evapd *in vacuo*, the residue dissolved in Me_2CO (5 ml) and reoxidized with a few drops of Jones' reagent. The product resulting after usual work up consisted of two compounds which were separated by prep. TLC (0.08 g on five 20 × 20 × 0.1 cm silica gel PF 254 + 366 plates with *n*-hexane–Et_2O–HCO_2H, 15:10:3). The lower band with the main compound yielded after extraction with Et_2O and crystallization from MeOH–H_2O **6** as needles mp 94–96° and

$$[\alpha]^{24}\ \frac{-35.6\ \ -37.4\ \ -43.4\ \ -74.9\ \ -90.6\ \ -116.1}{589\quad 578\quad 546\quad 436\quad 406\quad 366\ \text{nm}}$$

(MeOH; *c* 1.335). $C_{21}H_{36}O_5$ (368.25). IR ν_{max}^{KBr} cm^{-1}: 722, 780, 880, 960, 1010, 1032, 1082, 1094, 1132, 1190, 1218, 1270, 1320, 1370, 1400, 1460, 1700, 1750, 2930, 3140. ORD,

$$[\alpha]^{24}\ \frac{-37\ \ -56\ \ -112\ \ -262\ \ -131}{350\quad 300\quad 250\quad 222\quad 210\ \text{nm}}\ \text{(MeOH)}.$$

Me (−)-*dihydro-allo-pertusarate* (**7**). Prepared from **6** (0.08 g) with CH_2N_2 in Et_2O at 20° for 5 min. Needles mp 72–74° (from MeOH) and

$$[\alpha]^{24}\ \frac{-39.9\ \ -42.5\ \ -51.4\ \ -85.1\ \ -102.8\ \ -137.0}{589\quad 578\quad 546\quad 436\quad 406\quad 366\ \text{nm}}$$

($CHCl_3$; *c* 2.255). R_f 0.51 (silica gel PF 254 + 366, *n*-hexane–Et_2O–HCO_2H, 25:25:6). $C_{22}H_{38}O_5$ (382.52). IR ν_{max}^{KBr} cm^{-1}: 698, 770, 800, 884, 922, 950, 998, 1010, 1032, 1072, 1170, 1190, 1210, 1264, 1306, 1368, 1390, 1436, 1454, 1700, 1760, 2920. ORD,

$$[\alpha]^{24}\ \frac{-42\ \ -75\ \ -133\ \ -316\ \ -633\ \ -666}{400\quad 350\quad 300\quad 250\quad 230\quad 222\ \text{nm}}\ \text{(MeOH)}.$$

Me (−)-*dihydropertusarate* (**8**). Prepared from **1** (0.5 g) with CH_2N_2 in Et_2O at 20° for 10 min. Silklike needles mp 61–63° (from MeOH) and

$$[\alpha]^{24}\ \frac{-67.8\ \ -71.4\ \ -83.3\ \ -133.3\ \ -158.3\ \ -196.4}{589\quad 578\quad 546\quad 436\quad 406\quad 366\ \text{nm}}$$

($CHCl_3$; *c* 0.84). R_f 0.56 (silica gel PF 254+366, *n*-hexane–Et_2O–HCO_2H, 25:25:6). $C_{22}H_{38}O_5$ (382.52). IR ν_{max}^{KBr} cm^{-1}: 742, 790, 1014, 1038, 1048, 1072, 1098, 1138, 1166, 1216, 1258, 1290, 1344, 1384, 1412, 1440, 1470, 1734 (–CO_2Me, –COMe), 1780 (γ-lactone CO), 2990. UV λ_{max}^{MeOH} (log ε): 209 nm (2.35).

Me (−)-*neo-dihydropertusarate* (**9**) and *Me* (+)-2(R)-*methyl*-3-*carboxymethyl*-18-*oxo*-*nonadec*-3-*enoate* (**17**). A soln of Na (0.01 g) in MeOH (3 ml) was heated under reflux with **8** (0.115 g) for 2 hr, the solvent removed *in vacuo*, the residue acidified with 10% H_2SO_4 and extracted with Et_2O. To the Et_2O extract was added excess CH_2N_2–Et_2O soln and the solvent removed *in vacuo* after 5 min. The resulting oily mixture was separated by prep. TLC (0.096 g on five 20 × 20 × 0.1 cm silica gel PF 254 + 366 plates with *n*-hexane–Et_2O–HCO_2H, 6:4:1). The lower band yielded after extraction with Et_2O and crystallization from MeOH–H_2O **9** (0.036 g) as flat needles mp 42–44° and

$[\alpha]^{24}$	−27.0	−28.5	−33.5	−54.3	−65.9	−82.4
	589	578	546	436	406	366 nm

($CHCl_3$; *c* 1.82). R_f 0.54 (silica gel PF 254+366, *n*-hexane–Et_2O–HCO_2H, 25:25:6). $C_{22}H_{38}O_5$ (382.52). ORD

$[\alpha]^{24}$	−38	−50	−79	−158	−227	−205
	400	350	300	250	232	226 nm

(MeOH). IR ν_{max}^{KBr} cm^{-1}: 730, 748, 868, 960, 1010, 1026, 1060, 1100, 1120, 1182, 1202, 1276, 1302, 1332, 1366, 1404, 1436, 1460, 1712, 1752, 2930.

Me ester **7** (0.1 g) yielded on similar treatment a compound mp 44–46° (0.012 g), identical to **9**. The upper band of the prep. TLC gave after extraction with Et_2O **17** (0.023 g) as a colourless oil of

$[\alpha]^{24}$	+6.9	+7.6	+10.1	+16.8	+20.2	+32.9
	589	578	546	436	406	366 nm

($CHCl_3$; *c* 1.185).

Dithiospiroketal of (−)-*dihydropertusaric acid* (**11**). Prepared from **2** (1.5 g) by reaction with ethanedithiol (2.5 ml) and BF_3–Et_2O (5 ml) in HOAc (30 ml) at 20° for 24 hr. After usual work up and crystallization from MeOH rectangular plates (1 g) mp 112–114° and

$[\alpha]^{24}$	−62.0	−65.0	−74.7	−121.1	−141.8	−179.9
	589	578	546	436	406	366 nm

($CHCl_3$; *c* 1.445). $C_{23}H_{40}O_4S_2$ (444.68). IR ν_{max}^{KBr} cm^{-1}: 700, 722, 874, 930, 986, 1006, 1054, 1090, 1190, 1242, 1264, 1324, 1354, 1382, 1420, 1462, 1724, 2930, 3100. MS *m/z* (rel. int.): 426 $[M-H_2O]^+$ (5%), 424 (8), 398 (14), 385 (8), 339 (11), 330 (9), 241 (12), 145 (19), 134 (19), 131 (19), 121 (69), 119 ($[Me\text{–}\underset{S\ \ S}{C}\text{–}]^+$ (100). ORD

$[\alpha]^{24}$	−63	−83	−120	−153	−50	−100
	400	350	300	262	234	230 nm

(MeOH).

Dithiospiroketal of methyl (−)-*dihydropertusarate* (**12**). Prepared by reaction of **11** with CH_2N_2–Et_2O for 10 min. Oil. $C_{24}H_{42}O_4S_2$ (458.70). IR ν_{max}^{film} cm^{-1}: 740, 864, 938, 1010, 1050, 1080, 1100, 1196, 1244, 1270, 1338, 1372, 1454, 1724 (–CO_2Me), 1760 (γ-lactone CO), 2920. MS *m/z* (rel. int.): 458 ($[M]^+$, 20%), 443 (12), 427 (5), 399 (22), 241 (7), 119 $[Me\text{–}\underset{S\ \ S}{C}\text{–}]^+$ (100).

(−)-*Pertusaranic acid* (**13**). Prepared by heating **11** (1 g) with freshly prepared Raney-Ni (40 g) in EtOH (50 ml) under reflux for 4 hr. After usual work up and crystallization from MeOH plates (0.2 g) mp 131–132° and

$[\alpha]^{24}$	−78.5	−82.0	−93.1	−153.8	−184.6	−230.7
	589	578	546	436	406	366 nm

(MeOH; *c* 1.17). $C_{21}H_{38}O_4$ (354.51). IR ν_{max}^{KBr} cm^{-1}: 700, 722, 886, 924, 986, 1018, 1054, 1076, 1094, 1190, 1242, 1328, 1350, 1380, 1420, 1464, 1722, 2930, 3150. MS *m/z* (rel. int.): 353 $[M-H]^+$ (69%), 335 (29), 308 (76), 281 (62), 263 (39), 132 (100),), 114 (81). ORD

$[\alpha]^{24}$	−86	−117	−165	−212	−79	−149
	400	350	300	262	236	230 nm

(MeOH).

Me (−)-*pertusaranate* (**14**). Prepared by methylation of **13** with CH_2N_2–Et_2O at 20° for 10 min. After crystallization from MeOH needles mp 62–64° and

$[\alpha]^{24}$	−69.3	−72.8	−84.2	−131.5	−158.7	−195.6
	589	578	546	436	406	366 nm

($CHCl_3$; *c* 1.14). $C_{22}H_{40}O_4$ (386.54). IR ν_{max}^{KBr} cm^{-1}: 700, 740, 778, 790, 822, 856, 874, 892, 930, 946, 1012, 1050, 1076, 1096, 1130, 1170, 1198, 1250, 1300, 1336, 1384, 1430, 1460, 1710 (–CO_2Me), 1762 (γ-lactone CO), 2930. MS *m/z* (rel. int.): 368 $[M]^+$ (72%), 350 (15), 336 (19), 322 (22), 309 (83), 295 (65), 263 (15), 157 (61), 146 (100), 129 (96), 128 (87), 109 (56). ORD

$[\alpha]^{24}$	−50	−64	−92	−115	−18	−39
	400	350	300	262	234	230 nm

(MeOH).

(−)-*Dihydropertusaric acid oxime* (**15**). Prepared by reaction of **2** (0.1 g) with NH_2OH. HCl (0.1 g) in pyridine (1 ml) at 20° for 24 hr. After usual work up and crystallization from MeOH plates mp 98–99° and

$[\alpha]^{24}$	−73.3	−76.5	−86.5	−142.9	−170.8	−213.5
	589	578	546	436	406	366 nm

(MeOH; *c* 1.007). $C_{21}H_{37}NO_5$ (383.51). IR ν_{max}^{KBr} cm^{-1}: 702, 726, 880, 966, 990, 1020, 1110, 1208, 1252, 1310, 1334, 1370, 1390, 1424, 1474, 1740, 3000, 3200.

(−)-*Iso-muronic acid oxime* (**16**). Prepared by reaction of **3** (0.05 g) with NH_2OH. HCl (0.05 g) in pyridine (1 ml) at 20° for 24 hr. After usual work up and crystallization from MeOH needles mp 98–100° and

$[\alpha]^{24}$	−27.6	−28.6	−31.6	−49.5	−56.7	−65.6
	589	578	546	436	406	366 nm

($CHCl_3$; *c* 1.675). $C_{21}H_{35}NO_5$ (381.50).

(+)-2(S)-*Methyl*-3(R)-*carboxy*-1,4(S),18ξ-*trihydroxynonadecan*-12 → 1-*olide* (**18**). Prepared by reduction of **2** (0.2 g) with $NaBH_4$ (0.5 g) in EtOH (10 ml) at 20° for 24 hr. After usual work up and slow crystallization from MeOH–H_2O flat needles mp 55–57° and

$[\alpha]^{24}$	+5.4	+5.4	+5.4	+10.1	+12.2	+18.9
	589	578	546	436	406	366 nm

(MeOH; *c* 1.475). $C_{21}H_{40}O_4$ (356.53). IR ν_{max}^{KBr} cm^{-1}: 720, 848, 884, 936, 952, 1030, 1060, 1130, 1180, 1206, 1310, 1330, 1380, 1460, 1735, 2920, 3420. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1175, 1770, 3510, 3620. MS *m/z* (rel. int.): 356 $[M]^+$ (11%), 338 (21), 323 (25), 320 (41), 312 (21), 294 (53), 239 (30), 221 (35), 194 (26), 139 (54), 129 (100), 113 (77), 100 (96). ORD

$[\alpha]^{24}$	+19	+24	+30	+56	+75
	400	350	300	250	230 nm

(MeOH).

2,22-*Cyclo*-2-*ethyl*-3(S)-*methoxycarbonyl*-4(S)-*hydroxynonadecan*-1 → 4-*olide* (**19**). The pyrazoline Me ester **5** (0.05 g) was

dissolved in conc. H_2SO_4 (2 ml), the soln kept at 20° for 20 min, dil with H_2O (10 ml) and extracted with Et_2O. The residue after evapn of solvent was separated by prep. TLC (two 20 × 20 × 0.1 cm silica gel PF 254 + 366 plates with *n*-hexane–Et_2O–HCO_2H, 15:10:3). The lower band gave after extraction with Et_2O and crystallization from pentane **19** (0.01 g) prisms mp 52–54°·$C_{23}H_{38}O_5$ (394.53).

Me 20-methyl-allo-pertusarate (**20**). Prepared by heating **5** (0.05 g) at 140° for 15 min and separation by prep. TLC (two 20 × 20 × 0.1 cm silica gel PF 254 + 366 plates with *n*-hexane–Et_2O–HCO_2H, 15:10:3). The upper band yielded after extraction with Et_2O and crystallization from pentane **20** (0.04 g) plates mp 50–52°·$C_{23}H_{38}O_5$ (394.53).

Hydrogenation of (+)-protolichesterinic acid. Hydrogenation of (+)-protolichesterinic acid (4 g) mp 107–108° and $[\alpha]_D^{24}$ + 10.1 in HOAc (75 ml) with 10% Pd–C (1 g) was carried out under normal conditions. After usual work up the residue was methylated with CH_2N_2 in Et_2O and the mixture of Me (+)-lichesterinate, Me (+)-neo-dihydroprotolichesterinate and Me (+)-dihydroprotolichesterinate chromatographed on silica gel (130 g, with 5% H_2O) and eluted with *n*-hexane–Et_2O (9:1). The first fraction (500 ml) gave Me (+)-lichesterinate (1 g) as plates mp 53–54° (from MeOH) and $[\alpha]_D^{24}$ + 28.0 ($CHCl_3$, *c* 1.9), the second fraction (300 ml) Me (+)-neo-dihydroprotolichesterinate (0.45 g) as needles mp 33–35° (from MeOH) and $[\alpha]_D^{24}$ + 23.5 ($CHCl_3$; *c* 2.405) and the third fraction (600 ml) Me (+)-dihydroprotolichesterinate (0.88 g) as thin needles mp 53–55° (from MeOH) and $[\alpha]_D^{24}$ + 48.7 ($CHCl_3$; *c* 1.87).

Saponification of Me (+)-dihydroprotolichesterinate. Me (+)-dihydroprotolichesterinate (0.3 g) was heated with KOH (0.5 g) in MeOH (5 ml). The residue after usual work up had mp 96–98° and consisted according to TLC and optical rotation of $[\alpha]_D^{24}$ + 33.8 ($CHCl_3$; *c* 2.75) of 50% of (+)-dihydroprotolichesterinic acid and 50% (+)-neo-dihydroprotolichesterinic acid.

Me (−)-dihydro-allo-protolichesterinate. Prepared by hydrogenation of (−)-allo-protolichesterinic acid (0.2 g) mp 94–95° and $[\alpha]_D^{24}$ − 96.2 in HOAc (10 ml) with 10% Pd–C (0.1 g) under normal conditions for 2 hr. After usual work up and crystallization from Me_2CO (−)-dihydro-allo-protolichesterinic acid mp 110–120° and $[\alpha]_D^{24}$ − 41.6 (MeOH; *c* 1.385) was obtained containing according to TLC two minor products. The acid was methylated with CH_2N_2–Et_2O and gave after two crystallizations from MeOH pure Me (−)-dihydro-allo-protolichesterinate as silk-like needles mp 66–67° and

$[\alpha]^{24}$	−48.4	−50.2	−55.5	−96.5	−116.6	−150.5
	589	578	546	436	406	366 nm

($CHCl_3$; *c* 1.295). ORD

$[\alpha]^{24}$	−112	−202	−292	−720	−1575	−1417
	400	350	300	250	224	214 nm

(MeOH).

Analysis of P. ophthalmiza. (a) The lichen (9 g, Norway, Oppland, Lunner, S of Aurtjern, on bark of *Alnus incana*; leg. et det. T. Tønsberg, 12.9.1982, coll. no. 7535; voucher specimen in BG) was removed from the bark, extracted with Et_2O for 8 hr, the solvent removed *in vacuo* and the residue (0.7 g) chromatographed on silica gel (30 g, with 5% H_2O). *n*-Hexane–Et_2O (17:1) eluted a solid product which crystallized from $CHCl_3$–MeOH as plates mp 235–237° and $[\alpha]_D^{24}$ + 13.2 ($CHCl_3$; *c* 0.7345), identical with taraxerone. (b) The lichen (2 g, Norway, Oppland, Ringebu, on bark of *Salix caprea*, leg. et det. T. Tønsberg, 11.8.1984, coll. no. 9026; voucher specimen in BG) was worked up as described under (a) and the residue chromatographed on silica gel (5 g, with 5% H_2O); the column was eluted with an *n*-hexane–Et_2O gradient and gave four fractions which were analysed by MS.

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