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New tricycloalternarenes produced by the phytopathogenic fungus *Alternaria alternata*

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Abstract

We succeeded in the isolation and structure elucidation of nine novel tricycloalternarenes from the culture filtrate of the phytopathogenic fungus *Alternaria alternata*, isolated from *Brassica sinensis*. Tricycloalternarenes are closely related to ACTG-toxins. Structural differences mainly occur in the isoprenoid side chain and the substitution pattern of the C-ring of the tricycloalternarenes. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Alternaria alternata*; Fungi Imperfecti; Phytotoxins; Tricycloalternarenes; ACTG-toxins; Structural elucidation

1. Introduction

Molds of the genus *Alternaria* Nees ex Wallr. are widely distributed in many food and feed crops as well as in decaying organic material, household dust and soil and are well known as producers of substances with a great variety of differing structures. Lactones, perylenes, quinones, tetramic acids, decatrienoic acids, cyclic peptides, isoprenoid derivates and some others have different bioactivities. Many of these secondary metabolites act as phytotoxins which are either host-specific or nonspecific. Some compounds have additional antibacterial, antiviral, cytotoxic or insecticidal effects (Montemurro & Visconti, 1992). The species *Alternaria alternata* [Fries] Keissler is of particular scientific interest, because different pathotypes producing host-specific toxins exist, which are involved in phytopathogenic processes (Otani, Kohmoto & Kodama, 1995).

Recently we isolated a number of terpenoid compounds named tricycloalternarenes (TCAs) from the culture filtrate of a strain of *A. alternata* which originated from *Brassica sinensis* (Liebermann, Ellinger, Günther, Ihn & Gallander, 1997). The strain was used originally for the production of the nonspecific phytotoxin tentoxin (Liebermann & Oertel, 1983). These metabolites are closely related to the minor ACTG-toxins (*Alternaria citri* tangerine-toxins) which damage leaves of sensitive varieties of *Citrus reticulata*. These ACTG-toxins found in the tangerine pathotype of *A. alternata* (= *A. citri*) were regarded as host-specific at first (Kohmoto, Scheffer & Whiteside, 1979; Kono, Gardner, Suzuki, Kondo & Takeuchi, 1989; Kono, Gardner, Suzuki & Takeuchi, 1986). Later this view became rather doubtful (Gardner, Kono & Chandler, 1986; Kohmoto et al., 1993). Also in our view the existence of TCAs (partly identical with ACTG-toxins) in such a strain of *A. alternata* supports the opinion that these compounds are nonspecific toxins. At most they should be denoted as host-selective in the sense of Cooke, Jenkins and Lewis (1997).

In this paper we report the isolation and structure elucidation of further novel TCAs. Thus, altogether we found nineteen TCAs distinguishable above all by modified side chains and different substitution patterns in the C-ring.

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2. Results and discussion

The isolation of the new and especially of the hydrophilic TCAs became possible with the substitution of the former ether-extraction (Liebermann et al., 1997) by a solid-phase extraction. The residue was suspended in hot water. The water filtrate (hydrophilic TCAs) was subjected to a gel filtration on Sephadex G15, V_E/V_0 values are given in Table 2. Both the remainder, not soluble in water, and the hydrophilic substances were further separated by preparative HPLC runs. Rechromatography was almost always necessary. The concentration of TCAs varied up to 25 mg/l culture filtrate. Acidification of the culture filtrate caused an improvement of the yield of all TCAs for which chromatographic data are presented in Table 2.

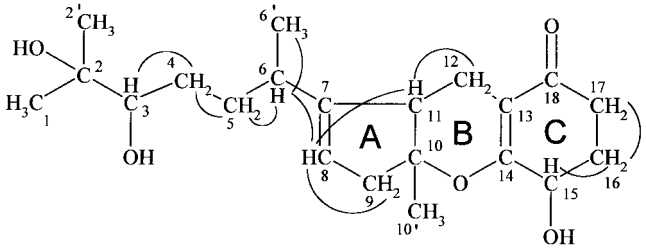
Connecting pairs could be isolated from many TCAs. They only differ in the position of their substituent at the ring C. Those TCAs, which carry the substituent in γ-position to the keto oxygen were named with the letter **a**. They are more hydrophilic in each case than the TCAs carrying the substituent in α-position. The different chromatographic behaviour of these closely related substances can be explained by a diminished polarity of TCAs **b** caused by the presence of a acyloin group. The existence of the TCA pairs should be due to a common precursor. Investigation of biosynthesis is underway.

The structures of TCAs **6a** and **6b** are derived from data Table 3. According to EIMS both compounds have the molecular formula of $C_{21}H_{32}O_5$. ^{13}C-NMR data confirmed the molecular formulae of each showing 21 resonances, four of which were due to methyls, six to methylenes, five to methines and six to quarternary carbons. ^{13}C-NMR data suggest that 29 of the 32 H-atoms are directly bonded to the C-atom. Therefore 3 hydrogens must be bonded as hydroxyl. The signals at δ_c 197.0 (TCA **6a**) respectively δ_c 197.6 (TCA **6b**) were each attributed to carbonyls, the fifth oxygen should be integrated in the ring system. The structure of both compounds was deduced unambiguously from analysis of DEPT, ^{1}H,^{1}H-COSY (Scheme 1), ^{1}H,^{1}H-TOCSY and ^{13}C,^{1}H-correlation (HMQC, HMBC). The numbering of C-atoms follows Kono et al., (1986). One of four methyls in the ^{1}H-NMR shows a doublet, which is due to the coupling with the proton at C-6 (δ_H 1.91). Further couplings are visible between this proton and methylenes CH_2-5 (δ_H 1.45). On the other hand allylic couplings are discernible between the proton at C-6 (δ_H 1.91) and the methine at C-8 (δ_H 5.31), from where couplings to C-9 and C-11 (allylic coupling) appear.

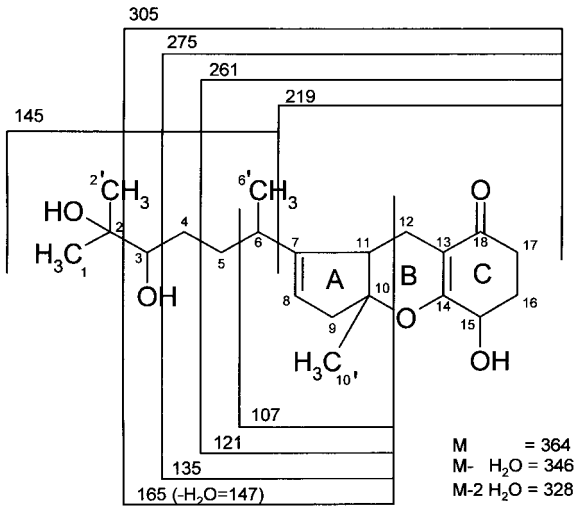
The position of the secondary hydroxyl at ring C is proved by HMBC. In TCA **6b** its position was assigned to C-17, whose proton (δ_H 4.00) gave a HMBC-cross-peak with the carbonyl at C-18 (δ_C 197.6). On the other hand TCA **6a** shows similar cross-peaks between the quarternary carbonyl at C-14 (δ_C 171.9) and the proton (δ_H 4.33) at C-15, where a hydroxyl of TCA **6a** is situated. The site of the secondary hydroxyl in the side chain was likewise ascertained by HMBC. The signals of the tertiary OH group were covered, therefore it was transformed with trichlorous acetylic isocyanate. After this conversion it could be recognized at C-2.

NMR concluded structures were supported by mass spectral fragmentation patterns. Three of five oxygens are neighbouring (m/z 219) (Scheme 2). Fragments (M-H_2O) and (M-2H_2O) were observed. A characteristic break goes through ring B. It has been based on a McLafferty-relocation.

Both TCAs **7a** and **7b** have the molecular formula $C_{22}H_{34}O_5$, indicating a methoxy group instead of a hydroxyl group in comparison with TCAs **6a** and **6b**. The change in the EIMS fragmentation pattern (m/z 319, 289, 275, 233 instead of 305, 275, 261, 219) showed the position of the methoxy in position at ring C. Its location at C-15 (TCA **7a**) respectively at C-17 (TCA **7b**) was verified by HMBC, analogous cross-peaks like in TCAs **6a** and **6b** were observed. Both



Scheme 1. Structure of TCA **6a**. Carbon numbers and visible COSY couplings are drawn in.



Scheme 2. EIMS of TCA **6a**-fragmentation pattern.

pairs of TCAs show the same allylic couplings between C-6 and C-8, therefore a double bond exists in ring A.

The TCAs **6a**, **6b**, **7a** and **7b** are the most hydrophilic ones compared to other TCAs. This is due to additional OH groups in their side-chain. This side-chain is well known from another phytotoxically effective substance (Suemitsu, Ohnishi, Morikawa, Ideguchi & Uno, 1994).

The mass spectrum of TCA **8a** is very similar to that of the known compound TCA **2a** (Liebermann et al., 1997). Both possess the same molecular formula ($C_{21}H_{30}O_4$). Nevertheless, a chromatographic separation of these TCAs by HPLC is possible. The differences in the EIMS fragmentation pattern of these TCAs showed the position of a double bond between C-5 and C-6 instead of the position between C-7 and C-8 (TCA **2a**). The position of this double bond was also shown in the ^{13}C-NMR (Table 3) and esp. by the homo- and heteronuclear correlations. The exact configuration in the side-chain of TCA **8a** was determined by NOESY experiments. Significant crosspeaks between the protons δ_H 1.60 (CH_3-6′) and 5.27 (HC-5) as well as δ_H 3.89 ($HOCH_2$-1) and 5.12 (HC-3) were detected, requiring structure shown in Table 1. A counterpart to TCA **8a** called TCA **8b**, could not be isolated in sufficient quantity for structure elucidation. However, it seems to exist, evidence is given by TLC.

A substance with a similar side-chain to TCA **8a** is TCA **9b**. It shows the molecular formula $C_{21}H_{30}O_3$. Twenty nine H-atoms are directly bound at carbon. Therefore this TCA can have only one hydrogen atom bound as a hydroxyl. The formula of TCA **9b** is shown in Table 1. Its structure was deduced of the NMR-data and HMBC/HMQC. The ^{13}C-NMR shows 4 methyls, no hydroxymethyl. The hydroxyl was located at C-17. The side chain configuration was likewise validated by NOESY.

Compound TCA **10b** was determined by NMR data only. The ^{13}C spectra (Table 3) reveal the presence of 4 groups of methyls, of which one appears as methoxy group. One shows up as doublet, that presupposes a single proton at C-6. NOESY couplings between the protons δ_H 5.22 (HC-3) and 3.93 ($HOCH_2$-1) ensure the trans configuration of the hydroxymethyl to the methin in the side chain. The methoxy in ring C is in α-position to the carbonyl, because HMBC-crosspeaks are seen between δ_H 3.61 (HC-17) and δ_C 195.9 (C=O).

In the TLC TCAs **11a** and **11b** showed the same colouring after detection like TCAs **1a** and **1b**. However, by comparison TCAs **11a** and **11b** were somewhat more hydrophobic in chromatographical systems. Because the colour in the TLC is based only on the structure of the side chain, it was assumed that these new TCAs differ in the ring system only. Therefore, it can be conjectured that in place of the hydroxyl a more hydrophobic methoxy exists like well known from the other TCAs. The matching molecule peak m/z = 362 was affirmed in the EIMS. Additionally, we find a fragment m/z = 233 of TCA **11a**. Such fragments are detectable in all TCAs which have a methoxy in ring C, in contradiction fragment m/z = 219 appears in a case of hydroxyl. Because the quantities of TCAs **11a** and **11b** were too small for NMR, the structures of these TCAs were suggested according EIMS data and chromatographical behaviour.

All the TCAs isolated by us so far are given in Table 1.

3. Experimental

The strain of *A. alternata* which was used has already been described elsewhere (Haenel, Liebermann, Brueckner & Troeger, 1985). For TCA-production the following liquid medium was used: KNO_3 9.0 g, $CaCl_2$ 0.8 g, KH_2PO_4 0.2 g, KCl 0.05 g, $MgSO_4{\cdot}7H_2O$ 0.15 g, $FeSO_4{\cdot}7H_2O$ 0.05 g, $ZnSO_4{\cdot}7H_2O$ 0.02 g, glucose 40.0 g, ad 1000 ml aqua dest. The pH was adjusted to 5.5 before autoclaving. Cultures were grown in 500 ml flasks filled with 100 ml medium. Culture duration amounted to 14 days at 28°C.

Culture filtrate was acidified HCl (pH < 3) and extracted over AMBERLITE XAD 16. Loaded XAD 16 was washed successively with 0.001 N HCl; 0.01 N NaOH and aqua dest. and eluted with PrOH-H_2O 1:1. Gel filtration (Sephadex G15) is described in (Liebermann & Oertel, 1983). TCAs **6a**, **6b**, **7a**, **7b** were separated preparatively by isocratic HPLC in MeOH-H_2O (13:7); **8a**, **11a**, **11b** in (3:1); the nonwater-soluble TCAs **9b**, **10b** in (17:3), conditions: Hibar Lichrosorb RP-18, 250 × 25 mm, 7 μm, guard column 30 × 16 mm, 10 μm, flow rate 5 ml/min. Analytical HPLC: 250 × 4 mm, 5 μm, guard column 30 × 4 mm, 7 μm, 0.6–0.8 ml/min. Detection at each case: 264 nm.

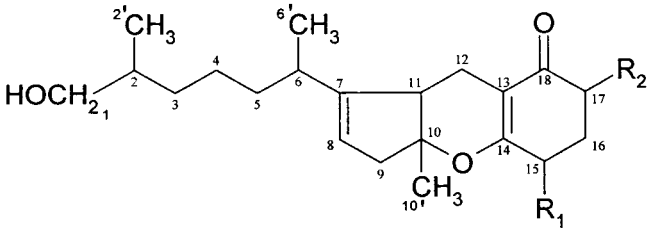
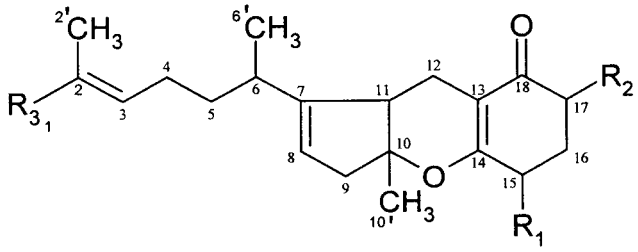
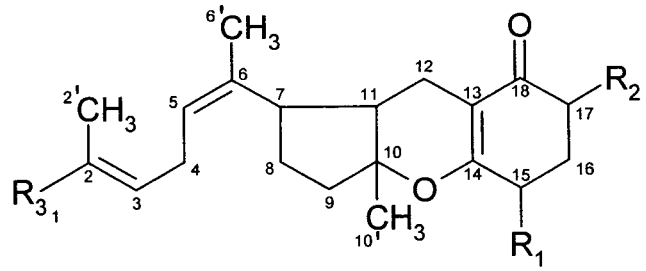
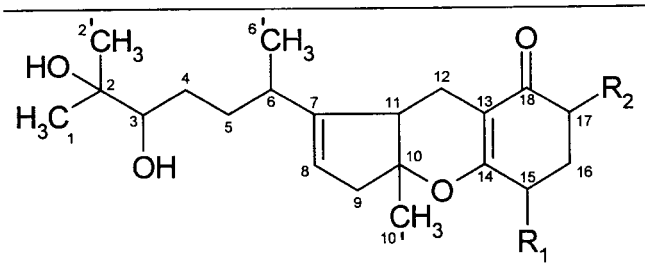
TLC (see Table 2) was done on Merck RP-18 F_{254} and Merck Kieselgel 60 F_{254}, respectively. Solvent I: EtOAc–Me_2CO-*n*-hexane (2:1:1); solvent II: EtOAc-MeOH–H_2O (100:4:1), solvent III: MeOH–H_2O (9:1). Detection: UV-quenching and anisic aldehyde spraying reagent.

NMR measurements were performed using a 5 mm probe head with a *z*-gradient (^{1}H 400 MHz, ^{13}C 100 MHz). EIMS were recorded at 70 eV.

3.1. Tricycloalternarene *6a*

UV λ_{max}^{MeOH} nm (log ε): 264 (4.10). ^{1}H and ^{13}C NMR: Table 3. EIMS 70 eV, m/z (rel. int.): 364.22439 ($C_{21}H_{32}O_5$ [M^+], 40), 346,21490 ($C_{21}H_{30}O_4$, 30), 328 (21), 305.17499 ($C_{18}H_{25}O_4$, 51), 287.16439 ($C_{18}H_{23}O_3$, 17), 27 (Kono, Gardner, Suzuki & Takeuchi, 1979), 16470 ($C_{17}H_{23}O_3$, 19), 261 (21), 248 (23), 223 (37), 219

Table 1
Survey of isolated TCAs. Newly obtained TCAs with asterisks, structure proposals with two astriks

	TCA **1a**: TCA **1b**: TCA **11 a****: TCA **11 b****:	R_1 = OH, R_2 = H R_1 = H, R_2 = OH R_1 = OCH_3, R_2 = H R_1 = H, R_2 = OCH_3
	TCA **2a**: TCA **2b**: TCA **3a**: TCA **3b**: TCA **4a**: TCA **4b**: TCA **10b***:	R_1 = OH, R_2 = H, R_3 = CH_2OH R_1 = H, R_2 = OH, R_3 = CH_2OH R_1 = OH, R_2 = H, R_3 = CH_3 R_1 = H, R_2 = OH, R_3 = CH_3 R_1 = OCH_3, R_2 = H, R_3 = CH_3 R_1 = H, R_2 = OCH_3, R_3 = CH_3 R_1 = H, R_2 = OCH_3, R_3 = CH_2OH
	TCA **5a**: TCA **5b**: TCA **8a***: TCA **9b***:	R_1 = OCH_3, R_2 = H, R_3 = CH_3 R_1 = H, R_2 = OCH_3, R_3 = CH_3 R_1 = OH, R_2 = H, R_3 = CH_2OH R_1 = H, R_2 = OH, R_3 = CH_3
	TCA **6a***: TCA **6b***: TCA **7a***: TCA **7b***:	R_1 = OH, R_2 = H R_1 = H, R_2 = OH R_1 = OCH_3, R_2 = H R_1 = H, R_2 = OCH_3

(35), 205 (31%), 187 (29), 165 (33), 147 (100), 145 (52), 135 (35), 133 (42), 121 (79), 107 (67); exact calcd mass for $C_{21}H_{32}O_5$: 364.22498 found 364.22439. IR: ν[cm^{-1}] = 545, 663, 754, 829, 924, 954, 1008, 1085, 1167, 1199, 1219, 1267, 1320, 1392, 1452, 1615, 2868, 2956, 3056, 3410.

Table 2
Chromatographic data of TCAs

TCAs	Gel filtration Sephadex G15 H_2O V_E/V_0	HPLC RP18 MeOH-H_2O (17:3) (0.8 ml/min) R_t [min]	HPLC RP18 MeOH-H_2O (13:7) (0.6 ml/min) R_t [min]	TLC solvent I silica gel R_f	TLC solvent II silica gel R_f	TLC solvent III RP 18 R_f
TCA 1a	3.5	4.60	18.00	0.42	0.46	0.51
TCA 1b	3.7	5.60	–	0.64	0.65	0.4
TCA 2a	3.5	4.40	15.40	0.43	0.47	0.60
TCA 2b	3.7	5.30	–	0.62	0.64	0.49
TCA 3a	not soluble	6.90	–	0.76	0.72	0.38
TCA 3b	not soluble	10.12	–	0.86	0.82	0.31
TCA 4a	not soluble	10.60	–	0.86	0.82	0.28
TCA 4b	not soluble	10.70	–	0.86	0.82	0.28
TCA 5a	not soluble	11.35	–	0.86	0.82	0.23
TCA 5b	not soluble	12.12	–	0.86	0.82	0.23
TCA 6a	2.06	–	7.60	0.22	0.25	0.70
TCA 6b	2.20	–	12.40	0.38	0.42	0.68
TCA 7a	2.10	–	14.84	0.26	0.30	0.65
TCA 7b	2.10	–	16.54	0.40	0.46	0.63
TCA 8a	3.26	–	15.20	0.43	0.47	0.60
TCA 9b	not soluble	8.30	–	0.86	0.82	0.35
TCA 10b	not soluble	6.30	–	0.70	0.68	0.39
TCA 11a	–	5.68	–	–	–	–
TCA 11b	–	5.92	–	–	–	–

3.2. Tricycloalternarene **6b**

UV λ_{max}^{MeOH} nm (log ε): 264 (4.16). ^1H and ^{13}C NMR: Table 3. EIMS 70 eV, m/z (rel. int.): 364.22580 ($C_{21}H_{32}O_5$ [M^+], 35), 346.21530 ($C_{21}H_{30}O_4$, 29), 328.20510 ($C_{21}H_{28}O_3$, 35), 305.17489 ($C_{18}H_{25}O_4$, 83), 288.17111 ($C_{18}H_{24}O_3$, 36), 287 (31), 275 (25), 261 (35), 248 (37), 221 (27), 219 (31), 205 (62), 187 (54), 147 (100), 145 (64), 135 (44), 133 (47), 121 (73), 107 (60); exact calcd mass for $C_{21}H_{32}O_5$: 364.22498 found 364.22580.

IR: ν[cm^{-1}] = 574, 686, 755, 830, 883, 914, 985,

Table 3
NMR data for TCAs **6a**, **6b**, **8a**, **9b** and **10b** in $CDCL_3$

Carbon No.	TCA **6a**		TCA **6b**		TCA **8a**		TCA **9b**		TCA **10b**	
	δ_C δ_H	DEPT	δ_C δ_H	DEPT	δ_C δ_H	DEPT	δ_C δ_H	DEPT	δ_C δ_H	DEPT
1	26.5 1.16 (s)	CH_3	26.5 1.14 (s)	CH_3	68.4 3.89/3.89 (m)	CH_2	25.6 1.64 (s br)	CH_3	68.4 3.93/3.93 (m)	CH_2
2	73.1 –	C	73.1 –	C	134.8 –	C	131.4 –	C	135.3 –	C
2′	22.9 1.10 (s)	CH_3	22.9 1.08 (s)	CH_3	13.7 1.53 (s br)	CH_3	17.6 1.52 (s br)	CH_3	13.7 1.57 (s br)	CH_3
3	78.1 3.24 (dd)	CH	78.2 3.23 (dd)	CH	124.3 5.12 (m)	CH	123.0 4.39 (m)	CH	124.6 5.22 (m)	CH
4	29.5 1.25/1.34 (m)	CH_2	29.5 1.25/1.34 (m)	CH_2	26.0 2.53/2.53 (m)	CH_2	26.4 2.51/2.51 (m)	CH_2	24.7 1.86/1.98 (m)	CH_2
5	31.8 1.45/1.45 (m)	CH_2	31.8 1.45/1.45 (m)	CH_2	126.6 5.27 (m)	CH	127.3 5.24 (m)	CH	34.5 1.30/1.43 (m)	CH_2
6	31.9 1.91 (m)	CH	31.9 1.91 (m)	CH	134.4 –	C	133.7 –	C	30.7 1.96 (m)	CH
6′	19.4 0.94 (d)	CH_3	19.4 0.92 (d)	CH_3	18.2 1.60 (s br)	CH_3	18.2 1.61 (s br)	CH_3	20.1 0.92 (d)	CH_3
7	150.3 –	C	150.3 –	C	41.5 2.70 (m)	CH	41.5 2.76 (m)	CH	150.3 –	C
8	120.0 5.31 (m)	CH	120.0 5.30 (m)	CH	25.1 1.75/1.61 (m)	CH_2	25.3 1.60/1.74 (m)	CH_2	119.6 5.28 (m)	CH
9	45.0 2.39/2.53 (m)	CH_2	45.0 2.39/2.53 (m)	CH_2	37.8 1.78/2.12 (m)	CH_2	37.8 1.79/2.10 (m)	CH_2	45.2 2.42/2.53 (m)	CH_2
10	88.6 –	C	88.6 –	C	87.7 –	C	87.3 –	C	88.6 –	C
10′	23.4 1.47 (s)	CH_3	23.4 1.42 (s)	CH_3	22.8 1.30 (s)	CH_3	22.4 1.30 (s)	CH_3	23.4 1.42 (s)	CH_3
11	46.6 2.73 (m)	CH	46.6 2.73 (m)	CH	43.2 1.88 (m)	CH	43.0 1.90 (m)	CH	46.8 2.75 (m)	CH
12	15.1 2.11/2.68 (m)	CH_2	15.1 2.11/2.68 (m)	CH_2	15.8 2.06/2.28 (m)	CH_2	15.8 2.08/2.38 (m)	CH_2	14.7 2.09/2.72 (m)	CH_2
13	106.2 –	C	105.2 –	C	107.5 –	C	105.0 –	C	106.4 –	C
14	171.9 –	C	172.9 –	C	167.7 –	C	170.0 –	C	171.5 –	C
15	66.5 4.33 (m)	CH	27.7 2.46/2.46 (m)	CH_2	66.1 4.37 (t br)	CH	27.8 2.46/2.46 (m)	CH_2	27.4 2.35/2.35 (m)	CH_2
16	28.8 1.93/2.17 (m)	CH_2	29.1 2.35/1.75 (m)	CH_2	28.9 1.97/2.20 (m)	CH_2	29.4 1.75/2.35 (m)	CH_2	27.5 1.80/2.12 (m)	CH_2
17	33.6 2.23/2.56 (m)	CH_2	71.0 4.00 (m)	CH	33.3 2.25/2.61 (m)	CH_2	71.1 4.04 (m)	CH	79.6 3.61 (m)	CH
17′									58.6 3.52 (s)	CH_3
18	197.0 –	C	197.6 –	C	198.1 –	C	189.2 –	C	195.9 –	C

1078, 1149, 1207, 1268, 1310, 1376, 1387, 1456, 1614, 2867, 2965, 3065, 3430.

3.3. Tricycloalternarene **7a**

UV λ_{max}^{MeOH} nm ($\log \varepsilon$): 264 (4.09). ^1H and ^{13}C NMR: resonances which differ from those of TCA **6a** (Table 3): δ_c 74.6 (C-15), 58.1 (C-15′), 26.9 (C-16), 32.3 (C-17), δ_H 3.72 (m, HC-15), 3.43 (s, H_3CO-15′), 1.99/2.04 (m, CH_2-16), 2.22/2.58 (m, CH_2-17). EIMS 70 eV, *m/z* (rel. int.): 378.23809 ($C_{22}H_{34}O_5$ [M^+], 31), 360.23120 ($C_{22}H_{32}O_4$, 31), 342.22030 ($C_{22}H_{30}O_3$, 27), 319,19119 ($C_{19}H_{27}O_4$, 62), 301 (17), 289 (23), 275 (39), 262 (37), 233 (29), 205 (35), 201 (42), 187 (48), 147 (100), 145 (71), 135 (56), 133 (54), 121 (100), 120 (73), 107 (87); exact calcd mass for $C_{22}H_{34}O_5$: 378.24063 found 378.23809. IR: ν[cm^{-1}] = 524, 764, 837, 893, 943, 1007, 1085, 1167, 1202, 1284, 1321, 1395, 1456, 1623, 2870, 2932, 2964, 3059, 3438.

3.4. Tricycloalternarene **7b**

UV λ_{max}^{MeOH} nm ($\log \varepsilon$): 266 (4.16). ^1H and ^{13}C NMR: resonances which differ from those of TCA **6b** (Table 3): δ_c 26.8 (C-15), 26.9 (C-16), 79.5 (C-17), 58.4 (C-17′), δ_H 2.31/2.42 (m, CH_2-15), 1.92/2.12 (m, CH_2-16), 3.61 (m, HC-17), 3.49 (s, H_3CO-17′). EIMS 70 eV, *m/z* (rel. int.): 378.24279 ($C_{22}H_{34}O_5$ [M^+], 28), 360 (16), 348.23059 ($C_{21}H_{32}O_4$, 92), 342 (15), 330.22039 ($C_{21}H_{30}O_3$35), 319.19110 ($C_{19}H_{27}O_4$, 94), 301 (23), 289 (19), 288.17199 ($C_{18}H_{24}O_3$, 33), 275 (17), 262 (17), 260 (24), 233 (21), 232 (19), 205 (43), 203 (50), 201 (35), 187 (35), 147 (88), 145 (58), 135 (48), 133 (42), 121 (75), 120 (100), 107 (54); exact calcd mass for $C_{22}H_{34}O_5$: 378.24063 found 378.24279. IR: ν[cm^{-1}] = 536, 777, 833, 878, 949, 1010, 1086, 1148, 1186, 1210, 1266, 1322, 1376, 1400, 1455, 1615, 2869, 2933, 2963, 3447.

3.5. Tricycloalternarene **8a**

UV $\lambda_{max}^{MeOH >}$ nm ($\log \varepsilon$): 262 (4.12). ^1H and ^{13}C NMR: Table 3. EIMS 70 eV, *m/z* (rel. int.): 346.21289 ($C_{21}H_{30}O_4$ [M^+], 7), 328.20379 ($C_{21}H_{28}O_3$, 46), 313.18039 ($C_{20}H_{25}O_3$, 6), 285 (6), 276.17291 ($C_{17}H_{24}O_3$, 40), 259 (10), 221.11749 ($C_{13}H_{17}O_3$, 44), 187.14979 ($C_{14}H_{19}$, 100), 145 (46), 135 (37), 121 (37), 107 (52); exact calcd mass for $C_{21}H_{30}O_4$: 346.21441 found 346.21289.

3.6. Tricycloalternarene **9b**

^1H and ^{13}C NMR: Table 3. EIMS 70 eV, *m/z* (rel. int.): 330.21960 ($C_{21}H_{30}O_3$ [M^+], 52), 287 (4), 261 (8), 248 (8), 237.11259 ($C_{13}H_{17}O_4$, 79), 221.11770 ($C_{13}H_{17}O_3$, 52), 203 (11), 189.16459 ($C_{14}H_{21}$, 100), 147 (50), 133 (21), 121 (21), 105 (60); exact calcd mass for $C_{21}H_{30}O_3$: 330.21950 found 330.21960.

3.7. Tricycloalternarene **10b**

^1H and ^{13}C NMR: Table 3.

3.8. Tricycloalternarene **11a**

EIMS 70 eV, *m/z* (rel. int.): 362.24429 ($C_{22}H_{34}O_4$, [M^+], 23), 344.23208 ($C_{22}H_{32}O_3$, 6), 332.23471 ($C_{21}H_{32}O_3$, 15), 262.15561 ($C_{16}H_{22}O_3$, 17), 251 (13), 235 (11), 233 (11), 225 (10), 207.17399 ($C_{14}H_{23}O_1$, 100), 201 (11), 189 (11), 177 (10), 133 (10), 121 (17), 108 (34), 107 (34); exact calcd mass for $C_{22}H_{34}O_4$: 362.24571 found 362.24429.

3.9. Tricycloalternarene **11b**

EIMS 70 eV, *m/z* (rel. int.): 362.24649 ($C_{22}H_{34}O_4$, [M^+], 66), 344.23431 ($C_{22}H_{32}O_3$, 2), 332.23471 ($C_{21}H_{32}O_3$, 87), 314 (11), 262 (6), 232 (15), 208.18150 ($C_{14}H_{24}O_1$, 100), 207 (49), 203 (17), 189 (15), 177 (15), 133 (8), 121 (15), 108 (42), 107 (26); exact calcd mass for $C_{22}H_{34}O_4$: 362.24571 found 362.24649.

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