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12-Carboxyretinoic Acids. Synthesis and Structure

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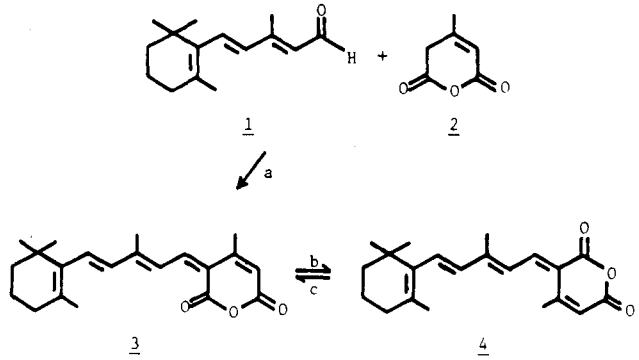
Four isomeric 12-carboxyretinoic acids and their dimethyl esters (trans, 13-cis, 11-cis, and 11-cis,13-cis), along with two isomeric retinoic anhydrides (13-cis and 11-cis,13-cis), have been prepared and fully characterized. The primary product of the base-promoted condensation of *trans*-(β-ionylidene)acetaldehyde and β-methylglutaconate has been shown to be the 11-cis,13-cis-diacid. The interconversions and relative stabilities of these retinoids are discussed in terms of their conformations.

The demonstrated prophylactic activity of retinoids vis-à-vis carcinogenesis[1] in epithelial tissues has prompted an interest in the preparation and chemical behavior of retinoid systems. In view of the relatively high activity and low toxicity of 13-*cis*-retinoic acid,[2-4] we were motivated to prepare a series of 13-cis-12-substituted retinoic acid derivatives with the potential of being cyclized to maintain a stable 13-cis double bond.

A system which aroused our interest was 12-carboxyretinoic acid. The electronegative substituent at C-12 was not expected to adversely affect the biological activity,[5] and perhaps more importantly, the literature regarding this system was contradictory and confusing.[6,7] In both of the reported cases it had been the authors' intention to synthesize vitamin A (*all-trans*-retinol) from (β-ionylidene)-acetaldehyde and β-methylglutaconic acid. As reported by Petrow and Stephenson,[6] the reaction of (β-ionylidene)acetaldehyde with diethyl β-methylglutaconate in methanolic potassium hydroxide gave an acid which they assigned as *all-trans*-12-carboxyretinoic acid, mp 203–205 °C. This acid could not be decarboxylated by heating in quinoline, and thus, this approach did not provide a route to vitamin A. On the other hand, Robeson and Cawley[7] reported that condensation of *trans*-(β-ionylidene)acetaldehyde with dimethyl β-methylglutaconate in methanolic potassium hydroxide gave a diacid (mp 192 °C) which decarboxylated readily to 13-*cis*-retinoic acid on being heated in quinoline in the presence of copper. Robeson and Cawley[7] did not fully assign the configuration of their diacid; they argued that the 11,12-double bond was cis on the basis of UV studies and analogies.

Petrow and Stephenson[6] had also synthesized another diacid. Starting from (β-ionylidene)acetaldehyde and β-methylglutaconic anhydride, they prepared an anhydride

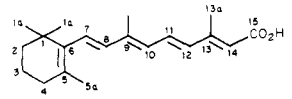
Scheme I[a]



[a] (a) Pyridine/THF. (b) *hν*. (c) Dark.

(1) Newton, D. L.; Henderson, W. R.; Sporn, M. B. *Cancer Res.* **1980**, *40*, 3413–3425.

(2) The numbering system used is an exemplified for *trans*-retinoic acid:



(3) Port, C. D.; Sporn, M. B.; Kaufman, D. G. *Proc. Am. Assoc. Cancer Res.* **1975**, *16*, 21.

(4) Sporn, M. B.; Squire, R. A.; Brown, C. C.; Smith, J. M.; Wenk, M. L.; Springer, S. *Science* **1977**, *195*, 487–489.

(5) Sporn, M. B.; Dunlop, N. M.; Newton, D. L.; Henderson, W. R. *Nature (London)* **1976**, *263*, 11–13.

(6) Petrow, V.; Stephenson, O. *J. Chem. Soc.* **1950**, 1310–1315.

(7) Robeson, C. D.; Cawley, J. D.; Weister, L.; Stern, M. H.; Eddinger, C. C.; Chechak, A. J. *J. Am. Chem. Soc.* **1955**, *77*, 4111–4119.

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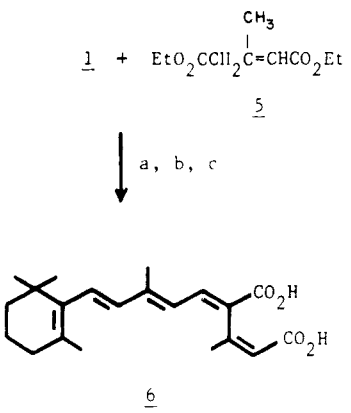
Scheme II[a]



[a] (a) KOH/MeOH. (b) H_2O. (c) KOH/EtOH.

of 12-carboxyretinoic acid (mp 126 °C) which upon saponification in aqueous medium gave a diacid which reverted to the anhydride upon warming and which they assigned as 13-*cis*-12-carboxyretinoic acid. This acid also failed to undergo decarboxylation.

In view of our interest in this series of diacids and of the literature discrepancies, we undertook a thorough study of this system.

Results

Condensation of *trans*-(β-ionylidene)acetaldehyde (**1**) with β-methylglutaconic anhydride (**2**), catalyzed by pyridine, in tetrahydrofuran at room temperature gave a dark red solid (mp 121–122 °C) which was clearly an anhydride of 12-carboxyretinoic acid (Scheme I). This anhydride, **3**, when exposed to light, gave an isomeric anhydride **4**, which reverted rapidly to **3** upon standing in the dark.

Replication of the Robeson and Cawley[7] condensation of *trans*-(β-ionylidene)acetaldehyde (**1**) with a mixture of *cis*- and *trans*-diethyl β-methylglutaconate (**5**) gave results in excellent agreement with the literature; i.e., a diacid (mp 192 °C) was obtained (**6**, Scheme II). Identical results were obtained when the reaction was carried out by substituting dimethyl β-methylglutaconate for the diethyl ester. This diacid was readily methylated to its dimethyl ester (**6e**) by treatment with diazomethane.

Treatment of the diacid **6** with anhydride-forming reagents such as dicyclohexylcarbodiimide, acetic anhydride, or trifluoroacetic anhydride in the cold gave anhydride **4**.

Treatment of the anhydride **3** with 8% hydrochloric acid in anhydrous methanol gave mainly a dimethyl ester of 12-carboxyretinoic acid (**7e**, Scheme III) which was distinctly different from **6e**. Diester **7e**, when treated with iodine in the dark, gave an isomeric diester, **8e**. Exposure of either **7e** or **8e** to light gave a fourth dimethyl ester, **9e**. These compounds were characterized by means of their ^{13}C and ^1H NMR parameters and specific proton–proton decoupling and NOE experiments. These data and the deduced structures are shown in Tables I–III.

Since we now had on hand four discrete dimethyl esters of 12-carboxyretinoic acid, it was of interest to consider whether all of them were formed in the condensation of *trans*-(β-ionylidene)acetaldehyde with the diesters of β-methylglutaconate, and only one was isolated due to the particular workup procedure, or whether, in fact, one isomer was preferentially formed. The Robeson and Cawley procedure[7] was therefore broken down into four separate parts: (i) condensation, consisting of mixing the substrates in methanolic potassium hydroxide and leaving the mixture at room temperature for 64 h; (ii) saponifi-

Table I. ^{13}C NMR Chemical Shifts of Retinoids[a]

retinoid		carbon																			
no.	assigned structure	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1a	5a	9a	13a	12a	OCH_3	OCH_3
	trans-retinoic acid	34.8	40.2	19.7	33.5	130.1	136.3	128.6	136.3	139.6	130.6	131.3	138.3	153.4	119.2		29.2	21.8	12.7	13.6			
	13-*cis*-retinoic acid	34.8	40.3	19.7	33.6	130.4	138.3	128.5	138.3	139.8	131.4	132.4	132.4	151.8	117.2		29.2	21.8	12.6	20.6			
3	13-*cis*-12-carboxyretinoic anhydride	34.9	40.5	20.0	34.1	133.8	138.5	136.0	138.5	155.0	128.2	144.2	118.6	155.4	113.8	160.4	29.4	22.1	13.0	19.2			
4	11-*cis*,13-*cis*-12-carboxyretinoic anhydride	35.1	40.5	19.9	34.1	133.7	138.6	136.2	138.2	154.9	126.7	145.1	119.8	153.7	116.2	163.8	29.3	22.0	12.8	23.4			
6	11-*cis*,13-*cis*-12-carboxyretinoic acid	34.8	40.2	19.7	33.5	130.5	138.3	130.4	138.1	144.0	125.6	133.1	132.9	152.3	120.9	166.5	29.1	21.8	12.6	25.3			
6e	dimethyl ester of **6**	34.8	40.2	19.7	33.5	130.7	138.2	130.7	138.0	144.4	125.3	133.0	132.5	152.0	120.7	166.1	29.2	21.8	12.6	25.3	165.4	51.0	51.8
7	13-*cis*-12-carboxyretinoic acid	35.0	40.6	20.2	33.8	132.3	138.8	129.7	139.5	142.5	128.1	135.1	130.3	154.4	119.9	167.0	29.3	22.0	12.2	26.6	166.4		
7e	dimethyl ester of **7**	34.7	40.1	19.7	33.5	130.7	138.3	130.3	138.8	143.9	126.9	135.5	130.5	153.4	119.0	165.5	29.1	21.8	12.1	26.2	165.9	51.2	50.9
8	*trans*-12-carboxyretinoic acid	34.7	40.1	19.7	33.5	130.6	138.3	130.4	138.3	143.6	126.8	131.5	136.6	152.5	118.1	167.5	29.1	21.8	12.4	15.7	168.5		
8e	dimethyl ester of **8**	34.7	40.1	19.7	33.5	130.7	138.3	130.7	138.3	144.0	126.5	131.7	136.3	151.9	117.8	168.2	29.1	21.8	12.5	15.5	166.9	51.8	51.0
9e	11-*cis*-12-carboxyretinoic acid dimethyl ester	34.7	40.0	19.7	33.5	130.7	138.3	131.3	138.1	145.6	125.3	135.1	134.1	153.1	121.1	166.5	29.1	21.8	12.8	19.7	166.5	51.9	51.0

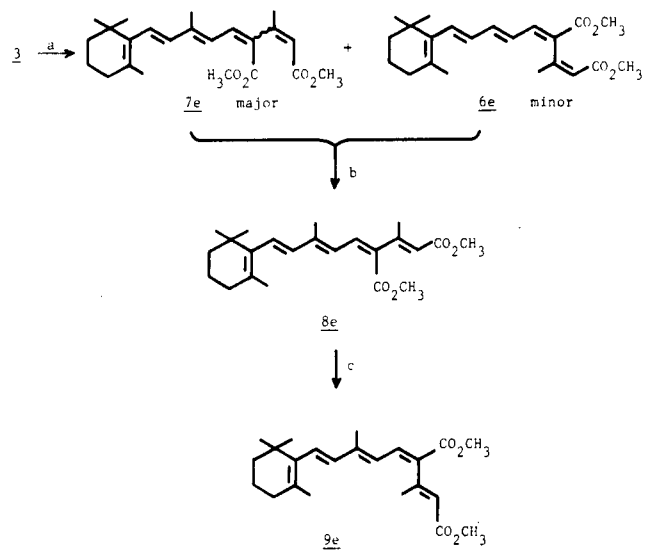
[a] In dioxane-d_8; shifts are given in parts per million.

Table II. ^{1}H NMR Chemical Shifts and Coupling Constants of Retinoids

retinoid		δ of H on carbon no.										J, Hz		
no.	assigned structure	7	8	10	11	12	14	1a	5a	9a	13a	7,8	10,11	11,12
	trans-retinoic acid[a]	6.23	6.09	6.14	7.02	6.32	5.72	0.94	1.62	1.94	2.25	16.1	11.9	15.1
	13-*cis*-retinoic acid[a]	6.23	6.13	6.17	7.00	7.73	5.58	0.94	1.62	1.93	2.00	15.9	11.5	15.4
3[b]	13-*cis*-12-carboxy-retinoic anhydride	6.80	6.40	7.63	7.72		5.97	1.03	1.73	2.19	2.22	15.0	12.0	
4[b]	11-*cis*,13-*cis*-12-carboxy-retinoic anhydride	6.80	6.40	6.93	8.18		5.90	1.06	1.75	2.20	2.40	15.0	13.0	
6[b]	11-*cis*,13-*cis*-12-carboxy-retinoic acid	6.36	6.15	5.93	7.41		5.90	0.97	1.65	1.99	1.99	16.0	12.0	
6e[a]	11-*cis*,13-*cis*-12-carboxy-retinoic acid dimethyl ester	6.35	6.10	5.98	7.42		5.88	0.93	1.60	1.94	1.97	16.2	12.5	
7[b]	13-*cis*-12-carboxy-retinoic acid	6.31	6.24	7.36	6.80		5.75	1.04	1.72	1.98	2.07			
7e[a]	13-*cis*-12-carboxy-retinoic acid dimethyl ester	6.35	6.15	7.18	6.86		5.75	0.95	1.64	1.94	2.03	16.6	11.6	
8[b]	*trans*-12-carboxy-retinoic acid	6.36	6.24	6.56	7.02		5.86	1.08	1.72	2.05	2.36			
8e[a]	*trans*-12-carboxy-retinoic acid dimethyl ester	6.36	6.12	6.26	7.07		5.63	0.94	1.61	1.99	2.29	16.2	12.5	
9e[a]	11-*cis*-12-carboxy-retinoic acid dimethyl ester	6.40	6.12	6.18	7.55		5.57	0.93	1.60	2.01	2.18	16.0	13.0	

[a] Acetone-d_6 solution, 250 MHz. [b] Dioxane-d_8 solution, 100 MHz.

Scheme III[a]



[a] (a) HCl/MeOH, 0 °C. (b) I_2 (dark). (c) $I_2/h\nu$.

Table III. Nuclear Overhauser Enhancements (%)[a,b] for Retinoids

retinoid		group irradiated	proton observed				
no.	assigned structure		14	11	10	8	7
	trans-retinoic acid	9-CH_3	0	26	0	0	
		13-CH_3	0	25	0	0	
	13-*cis*-retinoic acid	9-CH_3	0	26	0	0	
		13-CH_3	41	26	0	0	
3	13-*cis*-12-carboxy-retinoic anhydride	9-CH_3	0	25	0	0	22
		13-CH_3	31	26	0	0	0
6e	11-*cis*,13-*cis*-12-carboxyretinoic acid dimethyl ester	9-CH_3	0	30	0	0	15
		13-CH_3	30	0	0	0	0
7e	13-*cis*-12-carboxy-retinoic acid dimethyl ester	9-CH_3	0	27	0	0	18
		13-CH_3	30	14	0	0	0
8e	*trans*-12-carboxy-retinoic acid dimethyl ester	9-CH_3	0	24	0	0	18
		13-CH_3	0	21	0	0	0
9e	11-*cis*-12-carboxy-retinoic acid dimethyl ester	9-CH_3	0	25	0	0	15
		13-CH_3	0	0	0	0	0

[a] Degassed $(CD_3)_2CO$ solutions. [b] At 250 MHz.

cation by addition of aqueous potassium hydroxide, reflux, acidification, and extraction; (iii) purification by treatment of the product with potassium hydroxide in ethanol, separation of the insoluble potassium salt, acidification, and extraction; (iv) acidification of the filtrate of step iii and extraction. The condensation was carried out by using dimethyl β-methylglutaconate, and the material isolated at each of these steps was treated with excess diazomethane in order to convert any half-esters and/or diacids to their respective dimethyl esters. The products were then analyzed by HPLC; the results are shown in Table IV.

Since the starting (β-ionylidene)acetaldehyde contained a small amount of the cis isomer, the analogous condensation was carried out with *cis*-(β-ionylidene)acetaldehyde. The major product is indicated in Table IV.

The difference in stability of the diesters **6e–9e** in basic medium is shown in Table V. The 11-*cis*,13-*cis*-diester **6e** and the *trans*-diester **8e** saponified cleanly and rapidly without isomerization or decomposition in aqueous base at 60 °C. On the other hand, the 11-cis isomer **9e** was resistant to saponification, giving no diacid even after prolonged heating in basic medium. However, the diester **9e** did undergo slow isomerization mainly to the 11-*cis*,13-*cis*-diester **6e** which can saponify to **6** under these conditions. The 13-*cis*-diester **7e** was the most susceptible to both saponification and isomerization. In the presence of potassium hydroxide it saponified with some isomerization to the *trans*-diacid **8** but with sodium or lithium hydroxide saponification proceeded with isomerization to 11-*cis*,13-*cis*-diacid **6**.

Discussion

The easiest starting point for making stereochemical assignments of the retinoids **6–9** turned out to be the anhydride **3**. Its proton NMR spectrum (Table II) uniquely and unequivocally showed it to possess the 13-cis structure. Specifically, the presence of a very low field AB quartet

Table IV. Product of the Condensation of (β-Ionylidene)acetaldehyde with Dimethyl β-Methylglutaconate

config of (β-ionylidene)-acetaldehyde		product composition,[a] %						
						unknown		
		6e[b]	7e[c]	8e[d]	9e[e]	A	B	C
trans								
condensation[f]		85	5	1	0	7	1	0
saponification[g]		85	4	1	0	8	1	tr
purification:	solid[h]	93	0	0	0	4	3	tr
	filtrate[i]	18	21	9	0	47	0	5
cis[h]		51	0	0	0	49	tr	0

[a] HPLC after methylation with CH_2N_2; tr = trace amounts. [b] 11-*cis*,13-*cis*-12-Carboxyretinoic acid dimethyl ester. [c] 13-*cis*-12-Carboxyretinoic acid dimethyl ester. [d] *all-trans*-12-Carboxyretinoic acid dimethyl ester. [e] 11-*cis*-12-Carboxyretinoic acid dimethyl ester. [f] Mixing in KOH/MeOH for 64 h at ambient temperature. [g] Reflux in KOH/H_2O, acidification, and extraction. [h] Filtered off after KOH/EtOH. [i] Filtrate from solid.

(δ_A 7.72, δ_B 7.63; J = 12 Hz) can only be explained on the basis of H-10 being extremely deshielded (1.5 ppm from its position in retinoic acid) by the anisotropy of a proximate and coplanar carbonyl function; H-11 is also deshielded (0.7 ppm from its position in retinoic acid) due to the electronic effect of the 12-carboxy group. Thus, the observed signal enhancements (Table III) at H-11 upon irradiation of the methyl protons of C-13a and the similar enhancement at H-11 and H-7 resulting from irradiation of C-9a is testimony to the extended nature of the polyene chain.

The photoisomer of **3**, the anhydride **4**, can consequently, on the basis of its proton spectrum, be assigned the 11-cis,13-cis structure. In this case, H-10 is nowhere near the carbonyl and therefore appears at δ 6.93 ppm, being deshielded from its position in retinoic acid by 0.8 ppm due to the inductive effect of the 12-carboxy group. On the other hand, H-11 is now in the proximity of the 12-carboxy group and is therefore deshielded by the anisotropy of the coplanar carbonyl by 0.5 ppm from its position in the isomeric anhydride **3**.

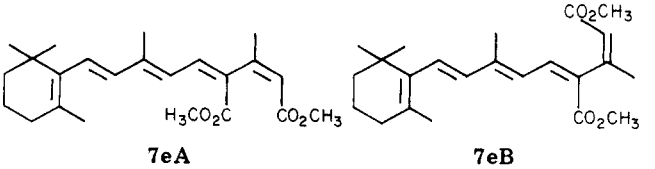
With these structure assignments in hand we may proceed to consider the ^{13}C chemical shifts (Table I) for these anhydrides since they provide a useful basis for configurational assignments in the diacids and their derivatives.

When comparing the ^{13}C chemical shifts of the 13-cis anhydride **3** to those of 13-*cis*-retinal or 13-*cis*-retinoic acid, we find that essentially all the vinyl resonances are shifted in a manner uniquely consistent with extended conjugation over the entire polyene chain. This is also borne out by the UV absorption [λ_{max} (EtOH) ~443 (ϵ ~26700)]. These shifts are particularly significant in view of the remarkable constancy of the chemical shifts in retinal isomers reported by Grant and Becker.[8]

Noteworthy are the chemical shifts of the C-13a methyl carbons. Grant and Becker[8] had found an 8-ppm upfield shift of the C-13a methyl resonance associated with a cis to trans configurational change at the 13,14 double bond. This was most likely due to a γ effect. They also indicated a 5-ppm upfield shift of the C-13a resonance to be associated with a cis to trans configurational change at the 11,12 double bond.[8] This observation is hard to account for and may be related to a γ effect due to general loss of coplanarity beyond C-12. In any event, it would appear that the difference between the chemical shift values for C-13a in **3** and **4** should be similarly related since the only difference between the structures lie in the configuration of the 11,12 double bond. In fact, the chemical shift of the 13a-methyl group in **3** is 19.2 ppm, in good agreement with the expected value of a 13-cis retinoid, and that of **4** is 4.2 ppm downfield at 23.4 ppm, in reasonable accord with expectation.

Since diacid **6** gave the anhydride **4** as the initial cyclization product when treated with anhydride-forming reagents, the configuration of its 11,12 double bond is undoubtedly cis. We thus agree with Robeson and Cawley's assignment[7] that the major product of the potassium hydroxide catalyzed condensation of *trans*-(β-ionylidene)acetaldehyde with β-methylglutaconate diester has 11-cis stereochemistry. Comparison of the ^{13}C NMR spectra of the diacid **6** and of the anhydride **4** strongly suggests the cis orientation for the 13,14 double bond, and this is confirmed by the observed NOE at H-14 upon irradiation of the 13a-methyl protons (Table III).[9] We consequently assign 11-cis,13-cis structure to the diacid **6**. This assignment is consistent with Robeson and Cawley's[7] observation that decarboxylation of their diacid gave neovitamin A acid (13-*cis*-retinoic acid).

It had been expected that ring opening of the anhydride **3** would lead to 13-*cis*-12-carboxyretinoic acid. In fact, this was the product reported by Petrow[6] to result from aqueous saponification of **3**. In our hands, careful saponification with aqueous potassium hydroxide gave a mixture of diacids, the composition of which changed with time under the reaction conditions. Due to the difficulties in separation and characterization of the components, methanolic saponification was examined. This led to the production of half-esters which turned out not to be useful for these studies. Success was achieved by submitting **3** to hydrolysis in anhydrous methanolic hydrochloric acid in the cold. The major reaction product was a dimethyl ester (**7e**) of 12-carboxyretinoic acid which was completely different from that obtained by esterification of diacid **6**. We were thus optimistic that we might have the 13-cis isomer in hand. Examination of the proton NMR spectrum served to confirm our expectation (Table II). The H-10 signal in this isomer appeared at substantially lower field (7.18 ppm) than that in the 11-*cis*,13-*cis*-dimethyl ester **6e** (5.98 ppm), consistent with a cisoid coplanar relationship between H-10 and the 12-carboxy group. To our surprise the ^{13}C NMR chemical shift of the 13a-methyl group was inconsistent with the value expected on the basis of comparison with known 13-cis retinoids. In fact, the observed chemical shift resembled that of **6e**. This apparent inconsistency was resolved by recognizing that the extended conformation of 13-*cis*-12-carboxyretinoic acid dimethyl ester (**7eA**) would involve severe steric strain



7eA **7eB**

between the 1,3-carbomethoxy groups, which could be relieved in a 12-s-cis conformation **7eB**. The existence of such a conformation in the new diester **7e** was confirmed by the low signal enhancement observed for H-11 upon irradiation of the C-13a protons[9] (Table III). The magnitude of the observed NOE suggests approximately equal conformational populations for **7eA** and **7eB**, and the

(8) Becker, R. S.; Berger, S.; Dalling, D. K.; Grant, D. M.; Pugmire, R. J. *J. Am. Chem. Soc.* **1974**, *96*, 7008–7014.

(9) Lewin, A. H.; Carroll, F. I.; Moreland, C. G. *J. Am. Chem. Soc.* **1981**, *103*, 6527–6529.

Table V. Saponification of 12-Carboxyretinoic Acid Dimethyl Esters

isomer of 12-carboxyretinoic acid dimethyl ester	reagent	temp, °C	12-carboxyretinoic acids formed,[a] %			
			11-*cis*,13-*cis* (6)	13-*cis* (7)	*trans* (8)	11-*cis* (9)
11-cis,13-cis (6e)	KOH	100	100	0	0	0
13-cis (7e)	KOH	100	0	75	25	0
	NaOH	100	35	50	15	0
	NaOH	40	20	80[b]	0	0
	LiOH	40	64	36[b]	0	0
trans (8e)	KOH	100	0	0	100	0
	NaOH	100	0	0	100	0
11-cis (9e)	KOH	100	22	0	4	0[c]
	NaOH	100	no reaction[d]			

[a] Analyzed by HPLC. [b] Undetermined amount of starting material also present. [c] 68% starting material also present. [d] A small amount of 11-cis,13-cis diester 6e was formed.

observation that neither cooling to −50 °C nor heating to +50 °C of the diester **7e** produced any changes in the chemical shifts or in the magnitude of the NOEs further supports a situation in which the conformations **7eA** and **7eB** are almost equal in energy. Because only a single set of resonances is observed, both in ^1H and ^{13}C NMR spectra, the two conformations must be in rapid dynamic equilibrium in the temperature range −50 to +50 °C, suggesting that the barrier to rotation between **7eA** and **7eB** must be very low.

The diesters **8e** and **9e** obtained by iodine-promoted isomerization of **7e** in the dark and under fluorescent lights were readily assigned as *all-trans*- and 11-*cis*-12-carboxyretinoic acid dimethyl esters, respectively, on the basis of the chemistry of their formation and of their NMR spectral parameters. Thus, **8e** has ^{13}C NMR signals at 12.5 and 15.5 ppm (Table I) in reasonable agreement with the values reported for the 9a- and 13a-methyl groups, respectively, in retinoic acid and some of its derivatives.[10] Similarly, the proton spectrum of **8e** displays methyl singlets at 1.99 and 2.29 ppm, in excellent accord with the values 1.94 and 2.25 ppm obtained for *trans*-retinoic acid. In addition, NOE enhancements (Table III) consistent with those obtained for *trans*-retinoic acid[9] and with those reported for *trans*-retinal[11] are observed for **8e**. Finally, the nearly identical UV parameters of **8e** and *trans*-methyl retinoate led to assignment of the trans configuration to **8e**.

The diester **9e** exhibits a 4.2-ppm downfield shift in the ^{13}C NMR resonance of the 13a-methyl group as compared to that for the diester **8e** (Table I). This is consistent with the 5-ppm chemical shift difference between the 13a-methyl groups reported for *trans*- and 11-*cis*-retinal.[8] Since irradiation at the frequency of the 9a- methyl proton resonance produces an NOE nowhere except at H-7 and H-11 (Table III), the stereochemistry from C-7 to C-11 must be trans; trans stereochemistry is similarly deduced from the lack of NOE at H-14 upon irradiation at the proton frequency of C-13a.

The unequivocal assignment of the 11-cis,13-cis configuration to the product obtained in the condensation of (β-ionylidene)acetaldehyde with β-methylglutaconate esters confirms the 11-cis assignment of Robeson and Cawley[7] and raises questions as to the reason that an apparently different product was obtained by Petrow and Stephenson[6] as well as to the identity of that product. Three possibilities come to mind, namely, that Petrow and Stephenson had isolated: (a) the *trans*-diacid, as they claimed, (b) the 11-*cis*-diacid, or (c) the 9-*cis*,11-*cis*,13-*cis*-diacid. If either the *trans*-diacid or the 11-*cis*-diacid were a primary reaction product, it could have been isolated by Petrow and Stephenson[6] but would have isomerized to the 11-cis,13-cis isomer during Robeson and Cawley's[7] purification procedure. Alternatively, since we had found that both the 13-*cis*- and *all-trans*-diester are capable of photochemical isomerization to 11-*cis*-diester, it is conceivable that an 11-*cis*-diacid would have been isolated in Petrow's laboratory. Both these possibilities were eliminated by our control experiments. The data in Table V demonstrate that neither *trans*-, 11-*cis*-, nor 13-*cis*-diester or -diacid is formed at any stage of the reaction sequence; therefore, the trans and 11-cis isomers are *not* primary reaction products. In addition, 11-cis product could not have been formed photochemically since the required precursors, trans or 13-cis isomers, were not formed (in the dark). Finally, if Petrow and Stephenson's (β-ionylidene)acetaldehyde had been predominantly the cis isomer, their product could have been 9-*cis*,11-*cis*,13-*cis*-diacid. However, this diacid has been reported by Robeson and Cawley (diacid D),[7] and the physical parameters do not seem to correspond to those of Petrow's acid. Thus, we cannot account for Petrow's reported result, but there is no doubt that the diacid formed in the condensation of *trans*-(β-ionylidene)acetaldehyde with either diethyl or dimethyl β-methylglutaconate, promoted by either sodium or potassium hydroxide, is 11-*cis*,13-*cis*-12-carboxyretinoic acid (**6**).

These results confirm that this Knoevenagel-like condensation leads to cis stereochemistry in the newly formed double bond. Although the conditions for the formation of the anhydride **3** are fairly different, it is nevertheless noteworthy that in this case the product has trans stereochemistry at C-11. However, it is possible that, in this case, **4** is the primary reaction product and isomerizes to **3** spontaneously in the dark.

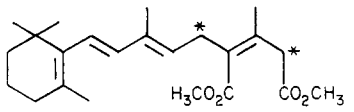
In view of the known propensity of potassium bases to cause isomerization in retinoid systems[12] and of the 13-*cis*-diester **7e** to isomerize (Table V), it seemed possible that the apparently unstable product mixture obtained from potassium hydroxide saponification of the anhydride **3** was due to isomerization. Therefore, the saponification of **3** was carried out by using aqueous sodium hydroxide, and, indeed, 13-*cis*-diacid **7** was the major (85%) product. It was accompanied by ca. 8% of 11-*cis*,13-*cis*-diacid **6** and an unidentified impurity. Treatment of the diacid **7** with potassium hydroxide led to its isomerization to the diacid **6**. These results prompted us to reinvestigate the condensation of *trans*-(β-ionylidene)acetaldehyde with *trans*- and *cis*-dimethyl β-methylglutaconate with sodium hydroxide replacing potassium hydroxide. Robeson and Cawley[13] had shown that the same product is obtained by

(10) Englert, G. *Helv. Chim. Acta* **1975**, *58*, 2367–2390.
(11) Rowan, R.; III; Warshel, A.; Sykes, B. D.; Karplus, M. *Biochemistry* **1974**, *13*, 970–980.
(12) French Patent 1 320 153.

starting with β-methylglutaconate of either stereochemistry. However, since they had used potassium hydroxide, reinvestigation seemed warranted. In fact, identical results were obtained whether the condensation of *trans*-(β-ionylidene)acetaldehyde was promoted with either sodium or potassium hydroxide. Thus, potassium ion is not a factor in this reaction, confirming that 11-*cis*,13-*cis*-12-carboxyretinoic acid (**6**) is the primary product of the condensation reaction.

The differences in stability of the diesters **6e–9e** (Table V) seem to suggest that the 11-cis,13-cis configuration represents an energy minimum vis-à-vis the trans, 13-cis, or 11-cis configurations. This result can be understood when the conformations of the isomeric diesters are considered. From the NMR and UV data it can be readily concluded that the *trans*-diester **8e** exists in an extended conformation with the carbomethoxy group at C-12 out of the polyene plane. Thus, inspection of the UV absorption maxima reveals that λ_{max} for the *trans*-diester **7e** is essentially identical with that of *trans*-methyl retinoate. It follows that there is no cross-conjugative contribution from the 12-carbomethoxy group. This is also confirmed by the proton chemical shifts of H-10 and H-14 in **8e** which are almost unchanged from those in *trans*-retinoic acid. By contrast, the 12-carbomethoxy group is conjugated with the C-7 to C-12 polyene moiety in the 11-*cis*,13-*cis*-diester **6e** as had been suggested by Robeson and Cawley.[7] The observed 20-nm bathochromic shift and the lack of a NOE from the 13a-methyl at H-10 support a conformation in which the 13,14 double bond is out of conjugation with the polyene. Essentially, the same conformation is deduced from the lack of NOEs in the 11-cis isomer, although somewhat more extended conjugation is suggested by UV. This places the 11-cis isomer in a unique position because base approach to the carbomethoxy group at C-14 in the 11-cis isomer is hindered by both the polyene π cloud and the 13a-methyl group, a situation which does not obtain in either of the other diesters. Consequently, the saponification rate of 11-*cis*-diester is very slow. The syn juxtaposition (syn strain) of the 13a-methyl group with the C-14 carbomethoxy group, coupled with the lack of conjugation past C-12, has a destabilizing effect on the 11-*cis*-diester relative to the 11-cis,13-cis and the all-trans isomers. Thus, whereas analogous truncated conjugation exists in the 11-cis,13-cis isomer and the trans isomer experiences syn strain, the 11-cis isomer contains both effects. It would follow that 11-cis isomer should isomerize to a lower energy form, either trans or 11-cis,13-cis. The observation that isomerization to the 11-cis,13-cis rather than to the trans isomer takes place suggests that the 11-cis,13-cis isomer is the lower energy configuration, implying that syn strain is more serious than loss of conjugation.

This conclusion may seem inconsistent with the lack of isomerization of the trans isomer. Namely, it might have been expected that isomerization to the lower energy 11-cis,13-cis configuration would take place. However, it needs to be borne in mind that the trans configuration is fully conjugated, and isomerization by deconjugation would represent a substantial energy barrier, particularly because it would most likely proceed in stepwise fashion, requiring the formation of a higher energy configuration, probably 13-cis, in an intermediate stage. In fact, the 13-*cis*-diester isomerizes to the 11-cis,13-cis isomer at 40 °C, consistent with the conclusion that 11-cis,13-cis is the lowest energy configuration. At 100 °C, however, isomerization of the 13-cis to the trans isomer takes place, consistent with providing more energy to an intermediate which could isomerize either way. Such an intermediate might be **10**,



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in which rotation about the 11,12 and 13,14 bonds should be equally likely, and the product composition would therefore be controlled by the relative energy of the isomers.

The *cis*-diester **7e** is the least stable; its high energy is manifest in its conformational heterogeneity. In the 12-s-cis conformer **7eB**, the polyene is deconjugated past C-12, but loss of energy is partially offset by conjugation with the 12-carbomethoxy group. This is confirmed by the downfield shift of H-10 (Table II) arising from the anisotropy of a coplanar and proximate carboxy group analogous to the downfield shift of H-12 in 13-*cis*-retinoic acid. Furthermore, the UV signal of **7e**, which is halfway between that of the 11-*cis*,13-*cis*-diester **6** and the *all-trans*-diester **8e**, speaks for the nearly equal populations of two conformations resembling **6e** and **8e**. The similarity between conformation **7eA** of the 13-*cis*-diester **7e** and the 11-*cis*,13-*cis*-diester **6e** is also suggested by the nearly identical magnitude of the NOEs observed for H-14 upon irradiation at the 13a-methyl resonance in 13-*cis*-12-carboxyretinoic acid dimethyl ester (**7e**) and 11-*cis*,13-*cis*-12-carboxyretinoic acid dimethyl ester (**6e**). The fact that this NOE is only 75% of the value expected for a completely planar system (as in 13-*cis*-retinoic acid) suggests that in order to obtain relief of steric strain, coplanarity of the 13,14 double bond with the 14-carbomethoxy group is relinquished in conformation **7eA** as well as in **6e**. However, since the population of **7eB** is only 50%, the distortion about the 13,14 double bond in **7eB** must substantially exceed the distortion in **6e**. This distortion may be responsible for the unexpectedly high ^{13}C NMR shift observed for the 13a-methyl group in **7e**. Once again, since the distortion in conformation **7eB** exceeds that in **6e**, the downfield shift of the 13a-methyl would be expected to be greater in **7eB** than in **6e**. However, since in **7e** it is averaged with the chemical shift of the 13a-methyl in **7eA**, the observed δ value happens to coincide with that of **6e**.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary-tube apparatus or on a Koffler hot stage, and they are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 267 grating spectrophotometer, ultraviolet spectra were recorded on a Cary 14 spectrophotometer, and mass spectra were obtained on an AEI MS-902 spectrometer. Proton NMR spectra were recorded on either a Varian HA-100 spectrometer or a Bruker WM-250 MHz spectrometer, and ^{13}C NMR spectra were determined on a JEOL JNM-PS-100 FT NMR instrument. Nuclear Overhauser enhancements (NOE) were determined on the Bruker WM-250 MHz spectrometer.

Analytical chromatography was carried out by using commerical silica gel F-254 for TLC and a Waters Associates high-pressure liquid chromatograph consisting of two constant-flow pumps (M6000A) controlled electronically by a solvent programmer (Model 660), a septumless nonstop-flow high-pressure injector (Model U6K), and a variable-wavelength UV detector (Model 450). The columns used were Waters Associates 3.9 mm × 30 cm μ-Porasil, μ-Bondapak C_{18}, and Radial Pak A and B cartridges in a Waters Associates radial compression module (Model 100).

Preparative separations were accomplished by using silica gel prepacked columns for medium-pressure liquid chromatography and two 10 mm × 25 cm Partisil 10 columns (packed at RTI) in

(13) Cawley, J. D. *J. Am. Chem. Soc.* **1955**, *77*, 4125–4129.
(14) Adams, R. A.; Van Duuren, B. L. *J. Am. Chem. Soc.* **1953**, *75*, 2377–2379.

series or a 4 mm × 25 cm column (packed at RTI) for high-pressure chromatography.

All laboratory operations involving retinoids and related polyene systems were performed under dim red lights and in an inert atmosphere.

11-*cis*,13-*cis*-12-Carboxyretinoic Acid (6). The procedure of Robeson and Cawley[7] was followed by using 777 mg (3.55 mmol) of *all-trans*-(β-ionylidene)acetaldehyde (1) and 727 mg (3.64 mmol) of a mixture (1:1) of *cis*- and *trans*-diethyl β-methylglutaconate.[15] The product, 397 mg of a yellow powder, was crystallized from acetone to give a pale yellow solid, mp 192 °C (lit.[7] mp 192 °C). Eventually absolute EtOH was found to be a better crystallization solvent. ^{13}C NMR suggested an 11-cis,13-cis structure for 6: UV (EtOH) λ_{max} 323 nm (ε 21 700); mass spectrum, calcd for $C_{21}H_{28}O_4$ *m/e* 344.1987, found 344.1983.

11-*cis*,13-*cis*-12-Carboxyretinoic Acid Dimethyl Ester (6e). A solution of 500 mg (1.51 mmol) of 11-*cis*,13-*cis*-12-carboxyretinoic acid (6) in 15 mL of MeOH and 10 mL of Et_2O was treated with CH_2N_2 (generated from 17 mmol of *N*-nitro-*N*-methyl-*N*-nitrosoguanidine) in the cold. Quenching with HOAc was followed by washing with Na_2CO_3, drying, and evaporaton. The residue, 550 mg, was packed onto a size C prepacked silica gel column and eluted with 50% Et_2O–hexane (2 mL/min, collecting 1-mL fractions). Monitoring by HPLC (μ-Porasil; 10% Et_2O–hexane, 2 mL/min; 365 nm) showed the desired product to be in fractions 162–240. Crystallization from hexane gave 170 mg of yellow crystals: mp 94–95 °C; UV (EtOH) λ_{max} 337 nm (ε 20 180); mass spectrum, calcd for $C_{23}H_{32}O_4$ *m/e* 372.2299, found 372.2304.

13-*cis*-12-Carboxyretinoic Anhydride (3). In a 25-mL, three-necked, round-bottomed flask equipped with magnetic stirrer, condenser, argon blanket, and injection inlet was placed 0.664 g (0.005 mol) β-methylglutaconic anhydride[14] in 5 mL of THF. The solution was cooled in an ice bath, and 1.08 g (0.005 mol) *all-trans*-(β-ionylidene)acetaldehyde was added, followed by the dropwise addition of 100 μL of pyridine. Monitoring by TLC (silica gel, 50% Et_2O–hexane) showed all the starting material to be consumed at the end of 1.5 h. The reaction mixture was thereupon diluted with Et_2O and dried over Na_2SO_4. Evaporation of the solvent, after filtration, gave a red gum. Treatment with hexanes at ~60 °C gave a red solution which deposited red crystals upon refrigeration. Filtration and drying gave red needles, mp 121–122 °C lit.[6] mp 126 °C). HPLC analysis (μ-Porasil; 5% MeOH in 9:1 hexane–Et_2O, 2 mL/min; 365 nm) showed the material to be quite impure. ^{13}C NMR suggested the presence of isomers. The material was purified by preparative HPLC. Analytical HPLC indicated it to be pure; proton and ^{13}C NMR spectra were also consistent with the presence of a single compound: UV (EtOH) λ_{max} 443 nm (ε 26 700); mass spectrum, calcd for $C_{21}H_{27}O_3$ *m/e* 326.1881, found 326.1886.

11-*cis*,13-*cis*-12-Carboxyretinoic Anhydride (4). (a) DCC. To a cold, stirred solution of 25 mg (0.073 mmol) of 11-*cis*,13-*cis*-12-carboxyretinoic acid in 2 mL dry THF in a 5-mL flask under N_2 was added 15.1 mg (0.073 mmol) dicyclohexylcarbodiimide. Monitoring by TLC (silica gel, 50% acetone–hexane) indicated the formation of anhydride. Solid was also visible in the flask (dicyclohexylurea). The solid was separated by filtration and washed with Et_2O. The combined filtrate and washes were washed with aqueous bicarbonate, dried over Na_2SO_4, and evaporated, leaving a red solid. HPLC [μ-Porasil; 5% MeOH (1:9 Et_2O–hexane), 2 mL/min; 440 nm] showed the major component to be the photoproduct of 13-*cis*-12-carboxyretinoic anhydride (3).

(b) Trifluoroacetic Anhydride. To a cold (0 °C), stirred solution of 50 mg (0.145 mmol) of 11-*cis*,13-*cis*-12-carboxyretinoic acid in 1.5 mL dry THF in a 5-mL round-bottomed flask equipped with an injection port and an argon blanket was added dropwise 23 μL (0.16 mmol) trifluoroacetic anhydride by syringe. After being stirred in the cold for a few minutes, the reaction mixture was diluted with 10 mL of Et_2O and washed with 5 mL of saturated aqueous $NaHCO_3$. After being dried (Na_2SO_4), the organic phase was evaporated. HPLC [μ-Porasil; 5% MeOH (1:9 Et_2O–hexane), 2 mL/min; 440 nm] showed a single peak coincident with that of the major photoproduct of 13-*cis*-12-carboxyretinoic anhydride. Within 1 h at room temperature, however, the only product detectable by HPLC was 3. Repetition of this experiment showed that the product does not survive for 1 h at 0 °C either. A ^{13}C NMR spectrum was obtained by dissolving 50 mg (0.145 mmol) of the diacid in 0.3 mL of THF-d_8 in an amber NMR tube and adding 46 μL (0.32 mmol) of trifluoroacetic anhydride. Control experiments (HPLC and ^{13}C NMR) had shown that 3 is stable to both trifluoroacetic anhydride and trifluoroacetic acid.

13-*cis*-12-Carboxyretinoic Acid Dimethyl Ester (7e). To an oven-dried, 1-L, round-bottomed flask under an argon atmosphere and equipped with a magnetic stirrer were added 450 mL of anhydrous MeOH, 55.5 mL of CH_3COCl (distilled), and then 1.5 g (4.6 mmol) of 13-*cis*-12-carboxyretinoic anhydride (3). After the mixture was stirred at low temperature for 10 days, TLC showed all the starting material to be consumed. The solution was then diluted with 400 mL of Et_2O and cooled to ca. 5 °C. Saturated Na_2CO_3 (930 mL) was then added dropwise, maintaining the temperature below 10 °C. The layers were separated, and the ethereal phase was dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, an oil (1.62 g) was obtained. Analysis by HPLC showed it to consist of a mixture of 12-carboxyretinoic acid dimethyl esters, mainly 13-cis (~60%) with less (~30%) 11-cis,13-cis. This mixture was chromatographed on a size C SiO_2 prepacked column, eluting with 25% Et_2O in hexane. From the column was obtained 129.1 mg of pure 13-*cis*-12-carboxyretinoic acid dimethyl ester. An additional 538 mg of less pure material was also obtained along with 26 mg of pure and 220 mg of less pure di-cis isomer. The HPLC-pure sample of the 13-*cis*-diester was crystallized from hexane to give 46 mg of pale yellow crystals: mp 57 °C; IR (KBr) 1720 cm^{-1}; UV λ_{max} 348 nm (ε 25 400); mass spectrum, calcd for $C_{23}H_{32}O_4$ *m/e* 372.2299, found 372.2304.

13-*cis*-12-Carboxyretinoic Acid (7). In a 500-mL round-bottomed flask was placed 3.65 g (1.2 mmol) 13-*cis*-12-carboxyretinoic anhydride (3) in enough THF to produce a clear solution; 73 mL of H_2O was added which caused the anhydride to come out of solution. More THF was added to dissolve the anhydride, and the solution was cooled to 0 °C; 56 mL of 1 N NaOH was then added. This caused the solution to lighten perceptibly and turn yellow. After the mixture was kept overnight at 0 °C, 400 mL of H_2O was added, and the solution was extracted with Et_2O (3 × 250 mL). The aqueous phase was acidified to pH 1 with 10% H_2SO_4 and extracted with ether (3 × 250 mL). The combined ether extracts were shaken with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and evaporated to yield 3.18 g (83% of theory) of a yellow powder.

For identification of this product, a small amount of the powder was dissolved in Et_2O and methylated with excess CH_2N_2/Et_2O. The reaction mixture was quenched with HOAc and washed with H_2O and saturated Na_2CO_3. HPLC analysis of the methylation product indicated ca. 85% 13-*cis*-12-carboxyretinoic acid dimethyl ester (7e), 8% 11-*cis*,13-*cis*-12-carboxyretinoic acid dimethyl ester (6e), and ca. 7% of an unknown. Attempted methylation with 8% HCl/MeOH led to isomerization.

Purification of the diacid was carried out via the di-*n*-butylamine salt. A solution of 800 mg (2.3 mmol) of the diacid 7 was dissolved in 80 mL of MeOH and treated with 0.46 mL (4.6 mmol) of *n*-butylamine. The solution was filtered into a dust-free Erlenmeyer flask and treated with 400 mL of filtered Et_2O. The resulting solution was cooled in an ice bath for 2 h and then stored at −16 °C. The first crop of crystals obtained (343 mg) had a melting point of 130 °C and was a single component (HPLC). This salt was dissolved in 200 mL of H_2O, and 10% H_2SO_4 was added dropwise until precipitation occurred. The solid was separated by filtration and dried under vacuum to yield 200 mg (83% of theory) of 13-*cis*-12-carboxyretinoic acid (7): mp 151 °C; IR (KBr) 1693 cm^{-1}; UV (EtOH) λ_{max} 314 nm (ε 25 466); mass spectrum, calcd for $C_{21}H_{28}O_4$ *m/e* 344.1987, found 344.1983.

***all-trans*-12-Carboxyretinoic Acid Dimethyl Ester (8e).** A solution of 1.6 g of the product of HCl methanolysis of 13-*cis*-12-carboxyretinoic anhydride (3) [see 13-*cis*-12-carboxyretinoic acid dimethyl ester (7e)] in 2.5 mL of Et_2O and 3.5 mL of hexane was treated with 0.5 mL of 1% I_2 in Et_2O and stored at 0 °C. The progress of the isomerization was followed by HPLC. After 6 days, another 0.5-mL aliquot of 1% I_2 in Et_2O was added, and the solution was kept at 0 °C for an additional 6 days. At this point HPLC analysis indicated only a small amount of 13-*cis*-diester

(15) Payne, G. B. *J. Org. Chem.* **1968**, *33*, 1284–1285.

Table VI. HPLC Retention Times for Retinoids

retinoid no.	retinoid assigned structure	column[a]	eluant[a]	retention time, min
3	13-*cis*-12-carboxyretinoic anhydride	B	E	3.4
4	11-*cis*,13-*cis*-12-carboxyretinoic anhydride	B	E	2.6
6	11-*cis*,13-*cis*-12-carboxyretinoic acid	A	C	11.6
6e	dimethyl ester of 6	B	D	8.2
7	13-*cis*-12-carboxyretinoic acid	A	C	9.2
7e	dimethyl ester of 7	B	D	5.0
8	*trans*-12-carboxyretinoic acid	A	C	5.8
8e	dimethyl ester of 8	B	D	3.2
9e	11-*cis*-12-carboxyretinoic acid dimethyl ester	B	D	3.6

[a] A, Radial Pak A; B, Radial Pak B; C, 3:1 CH_3CN/1% NH_4OAc in H_2O (solvent X) and H_2O (solvent Y), linear gradient of 37–50% X over 4 min; D, 5% Et_2O/hexane; E, 4% MeOH in 9:1 hexane/Et_2O.

7e to remain, so the solution was diluted with 280 mL of hexane and filtered through a layer of sodium thiosulfate on an asbestos filter into an ice-cooled receiver. The filtrate was evaporated, and the residue was loaded onto a 10-g silica gel 60 cleanup column. Elution with 1:1 Et_2O–hexane gave 1 g of an oil, which contained the desired material (HPLC). Chromatography on a size C SiO_2 prepacked column, eluting with 15% Et_2O in hexane, gave 127 mg of *all-trans*-diester **8e**, 38 mg of *all-trans*-diester **8e** contaminated with 11-*cis*-diester **9e**, 121 mg of 13-*cis*-diester **7e**, and 547 mg of later eluting material. The *trans*-diester **8e** was recrystallized from hexane to give 113 mg of pure *all-trans*-12-carboxyretinoic acid dimethyl ester: mp 85–86 °C; IR 1710 cm^{-1}; UV (EtOH) λ_{max} 358 nm (ϵ 28 800); mass spectrum, calcd for $C_{23}H_{32}O_4$ *m/e* 372.2299, found 372.2304.

***all-trans*-12-Carboxyretinoic Acid (8).** In a 5-mL Reacti-Vial was placed 175 mg (0.51 mmol) of *all-trans*-12-carboxyretinoic acid dimethyl ester (**8e**) in 0.35 mL of MeOH, 0.56 mL of 3 M NaOH in MeOH and 1.12 mL of 2.5 M aqueous NaOH. The vial was heated at 100 °C with stirring. After 2 h, TLC analysis showed the reaction to be complete; therefore, 10 mL of H_2O was added, and the reaction mixture was washed with Et_2O (3 × 10 mL), neutralized with NH_4Cl, and again washed with Et_2O (3 × 10 mL). The aqueous phase was acidified with 10% H_2SO_4 and extracted with Et_2O (3 × 10 mL). The ether extract was dried over Na_2SO_4, filtered, and evaporated to give a microcrystalline solid. HPLC analysis showed two very minor impurities. Recrystallization from absolute EtOH gave 122 mg (77%) of *trans*-12-carboxyretinoic acid (**8**): mp 200 °C; IR (KBr) 1685 cm^{-1}; (EtOH) λ_{max} 361 nm (ϵ 35 556); mass spectrum, calcd for $C_{21}H_{28}O_4$ *m/e* 344.1987, found 344.1988.

11-*cis*-12-Carboxyretinoic Acid Dimethyl Ester (9e). To a solution of 372 mg of the HCl methanolysis product of 13-*cis*-12-carboxyretinoic anhydride (**3**) [see 13-*cis*-12-carboxyretinoic acid dimethyl ester (**7e**)] in 1.6 mL of hexane was added 0.5 mL of 1% I_2 in Et_2O, and the resulting solution was left under fluorescent lights for 4 days. HPLC analysis indicated a 1:1 ratio between the *all-trans*-**8e** and 11-*cis*-diester **9e**. The solution was filtered through a bed of sodium thiosulfate and evaporated. The residue, 300 mg, was applied to a size C SiO_2 prepacked column and eluted with 10% Et_2O–hexane to give 61 mg of *all-trans*-diester **8e** and 91 mg of 11-*cis*-diester **9e**. Attempts to crystallize 11-*cis*-12-carboxyretinoic acid dimethyl ester (**9e**) failed: IR 1710 cm^{-1}; UV (EtOH) λ_{max} 347 nm (ϵ 23 500); mass spectrum, calcd for $C_{23}H_{32}O_4$ *m/e* 372.2299, found 372.2304.

Stepwise Investigation of the Robeson and Cawley Condensation of *trans*-(β-Ionylidene)acetaldehyde with Diethyl β-Methylglutaconate. To a 25-mL round-bottomed flask equipped with magnetic stirrer and maintained under N_2 were added 660 mg (3.0 mmol) of *trans*-(β-ionylidene)acetaldehyde and 516 mg (3.0 mmol) of diethyl β-methylglutaconate in 3.1 mL of 3 M methanolic KOH, and the reaction mixture was stirred at room temperature for 64 h. A quarter of the reaction mixture was withdrawn and treated with saturated aqueous NH_4Cl until the pH reached 8. The solution was then extracted with Et_2O (3 × 5 mL), and the combined ether phases were dried and concentrated to give 144 mg of an oil which was methylated with CH_2N_2 (see below). The remainder of the reaction mixture was treated with 4 mL of 3 M aqueous KOH and the solution refluxed for 30 min. At the end of this time 5 mL of H_2O was added, and the solution was extracted with Et_2O (2 × 10 mL). The aqueous layer was then treated with 12.5 mL of 10% H_2SO_4 and 10 mL of H_2O and extracted with Et_2O (4 × 20 mL). The extract was dried over Na_2SO_4, and one-third was removed and evaporated, giving 171 mg of a solid which was methylated with CH_2N_2. The remainder was evaporated to yield 447 mg of a solid which was dissolved in 3.5 mL of EtOH and 14 mL 10% KOH/EtOH. This was stirred at room temperature for 1.5 h. The solid which precipitated was filtered and washed with cold EtOH and cold Et_2O. It was then suspended in 0.5 mL of MeOH and 10 mL of Et_2O and treated with 4 mL of 10% H_2SO_4. The ether extract was washed with H_2O (2 × 5 mL), dried, and evaporated to give 161 mg of a solid which was methylated with CH_2N_2. The filtrate was treated with 5 mL of H_2O, and the pH was brought to 1 with 10% H_2SO_4. The solution was then extracted with Et_2O (2 × 15 mL), and the extract was washed with H_2O (3 × 20 mL), dried, and evaporated. The residue, 161 mg, was methylated with CH_2N_2.

Methylation Procedure. In each case CH_2N_2/Et_2O was generated from *N*-methyl-*N*-nitroso-*N*-nitroguanidine, and the material to be methylated (in a minimum of MeOH) was treated with an eightfold excess. The reaction mixture was stirred for 5 min, quenched with HOAc, and washed with 15 mL of H_2O, saturated aqueous Na_2CO_3 (2 × 10 mL), and H_2O (2 × 15 mL). The organic phase was dried, evaporated, and analyzed by HPLC (Table IV).

Condensation of *cis*-(β-Ionylidene)acetylaldehyde with Dimethyl β-Methylglutaconate. In a scintillation vial were placed 23 mg (0.12 mmol) of *cis*-(β-ionylidene)acetaldehyde, 18.5 mg (0.12 mmol) of dimethyl β-methylglutaconate, 1 mL of MeOH, and 0.1 mL of 3 M KOH/MeOH. The mixture was allowed to stand at room temperature for 64 h. It was then treated with saturated aqueous NH_4Cl to pH 8 and extracted with Et_2O (3 × 5 mL). The Et_2O extract was dried and evaporated to give 29 mg of product which was methylated and analyzed by HPLC (Table IV).

NaOH-Promoted Condensation of *trans*-(β-Ionylidene)-acetaldehyde with Dimethyl β-Methylglutaconate. In a 1-mL Reacti-Vial were combined 5 mg (0.02 mmol) of *trans*-(β-ionylidene)acetaldehyde, 4.1 mg (0.02 mmol) of dimethyl β-methylglutaconate (cis/trans mixture), 14 μL of MeOH, and 25 μL of 3 M NaOH/MeOH, and the mixture was kept at room temperature. After 64 h, 50 μL of 2.5 M NaOH/H_2O was added and the vial placed in a 100 °C sand bath for 1 h. The reaction mixture was then acidified with 10% H_2SO_4 and extracted with Et_2O. The product analyzed by HPLC was >98% 11-*cis*,13-*cis*-12-carboxyretinoic acid with ca. 1% of the 13-cis isomer and trace amounts of the trans isomer.

HPLC Analysis. The best separations were achieved by using Radial Pak cartridges, although stainless-steel-packed columns gave similar results. The anhydrides were analyzed by using Radial Pak B with 4% MeOH in 9:1 hexane/Et_2O (2 mL/min) as the eluant; detection was at 440 nm. For analysis of the diacids a Radial Pak A cartridge was used. The eluant was 3:1 CH_3CN/1% aqueous NH_4OAc (solvent X) and H_2O (solvent Y) with a linear gradient of 37–50% X at a 2 mL/min flow rate. Detection was at 350 nm. The diesters were analyzed on Radial

Pak B with 5% Et_2O in hexane at 2 mL/min as the eluant and with the UV detector at 350 nm. The retention data are shown in Table VI. The solvents were from Burdick and Jackson, and they were degassed prior to use.

Nuclear Overhauser Effect (NOE) Determinations. All ^1H NOEs were measured on a Bruker WM-250 spectrophotometer operating in the pulse-FT mode. Each sample (ca. 5 mg) was dissolved in 0.5 mL of dry deuterioacetone (Merck acetone-d_6 "100%"), degassed by several freeze–pump–thaw cycles and sealed. The NOE values were determined from the ratio between the relative peak intensities with the irradiating field on resonance and off resonance for the saturated ^1H signal. At least five NOE measurements were made for each ^1H signal of each sample studied. The standard deviation was always 2% or less. The field strength of the irradiating field was determined for each sample by setting the decoupling power level so that a maximum increase in intensity of interacting protons was obtained without affecting other proton signals. In all experiments the pulse delay time used was sufficiently long to allow complete recovery of all signals. This time ranged from 40 to 60 s, depending on the sample.

The ambient probe temperature was 24 °C; the high-temperature experiments were run at 50 °C and the low temperature experiments at −50 °C. No significant changes were observed in the spectra or the NOE values at either the high or low temperatures.

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Registry No. *trans*-1, 3917-41-7; *cis*-1, 56013-13-9; 2, 67116-19-2; 3, 81121-53-1; 4, 81177-15-3; *cis*-5, 73192-75-3; *trans*-5, 73178-43-5; 6, 81176-73-0; 6e, 80009-89-8; 7, 81176-74-1; 7e, 80040-38-6; 8, 6703-19-1; 8e, 79985-66-3; 9e, 80009-88-7; *cis*-dimethyl β-methylglutaconate, 1712-35-2; *trans*-dimethyl β-methylglutaconate, 41527-39-3; *trans*-retinoic acid, 302-79-4; 13-*cis*-retinoic acid, 4759-48-2.

Antineoplastic Cyclic Peptides from the Marine Tunicate *Lissoclinum patella*

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The tunicate *Lissoclinum patella* produces a family of lipophilic cyclic peptides all of which contain an unusual fused oxazoline–thiazole unit. The structures of three of these peptides, patellamides A–C have been determined by chemical and spectral methods. The patellamides are cytotoxic, exhibiting IC_{50} values of 2–4 μg/mL against L1210 murine leukemia cells. Additionally, patellamide A was active against the human ALL cell line CEM with an ID_{50} of 0.028 μg/mL. Ulithiacyclamide, a peptide previously reported from *L. patella* was also tested for cytotoxicity and exhibited 50% inhibition at doses of 0.35 and 0.01 μg/mL for the L1210 and CEM tests, respectively.

As part of a program to isolate antineoplastic natural products from marine invertebrates, we have undertaken a systematic study of didemnid tunicates from Palau of the Western Caroline Islands. The didemnids seemed likely candidates for several reasons. It is well-known that tropical didemnid species harbor unicellular prokaryotic algae,[1,2] and even though nitrogen fixation is yet to be demonstrated in these symbionts, we felt the possibility warranted an investigation for novel nitrogenous metabolites. This was particularly intriguing in view of the activities encountered with terrestrial alkaloids.[3] Furthermore, cytotoxicity has been documented in extracts of tunicates,[4] and cytotoxic constituents have been isolated by Rinehart,[5] Fenical,[6] and Howard.[7]

As a result of preliminary studies, we recently reported the isolation of *N,N'*-diphenethylurea (1; see Chart I) from *Didemnum ternatanum*[8] and the cyclic peptides ulithiacylamide (2) and ulicyclamide (3) from *Lissoclinum patella*.[9] Both tunicates were collected on reef flats near Korror Island, Palau Islands. We now report the isolation of three additional cyclic peptides, patellamides A (4), B (5) and C (6) from *L. patella* collected at Eil Malk Island, Palau Islands. All six of these metabolites were tested for antitumor activity against L1210 murine leukemia cells cultured in vitro. As depicted in Table I, ulithiacyclamide (2) was the most potent having an IC_{50} of 0.35 μg/mL, whereas ulicyclamide (3) had an IC_{50} of 7.2 μg/mL. Patellamides A (4), B (5), and C (6) exhibited approximately equal activities with IC_{50} values of 3.9, 2.0, and 3.2 μg/mL,

Table I. Cytotoxicity Testing Results for Metabolites 1-6 from Marine Tunicates

compd	IC_{50}, μg/mL L1210	CEM
N,N'-diphenethylurea (1)	>10	
ulithiacyclamide (2)	0.35	0.01
ulicyclamide (3)	7.2	
patellamide A (4)	3.9	0.028
patellamide B (5)	2.0	
patellamide C (6)	3.2	

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