Tetrahedron. Vol. 25, pp. 3341 to 3357. Pergamon Press 1969. Printed in Great Britain

THE CHEMISTRY OF STEROID ACIDS FROM *CEPHALOSPORIUM ACREMONIUM*[1]

T. S. CHOU[2,*], E. J. EISENBRAUN and R. T. RAPALA

Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma 74074, and The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206

(*Received in USA* 27 *December* 1968; *Received in the UK for publication* 17 *March* 1969)

Abstract—Cephalosporin P_1 (**1a**), isocephalosporin P_1 (**1c**) and monodesacetyl cephalosporin P_1 (**2a**) have been isolated from the organic extract of the fermentation broth of *Cephalosporium acremonium*. The acids **1a** and **2a** were obtained in 18% and 3% yield, respectively, from the crude concentrated butyl acetate extract. The structure and stereochemistry of **1a**, **1c**, and **2a** were established by chemical and spectral studies. Compound **1a**, **1c**, and **2a** are steroid acids with a *trans–syn–trans* B-boat conformation, each possessing three angular Me groups and one Me group attached to a secondary C atom. The placement and stereochemistry of the two OAc groups and two OH groups of **1a** and **1c** and also one OAc groups and three OH groups of **2a** are established. Additional data are presented which establish the side-chain structure at C-17 position and confirm the *trans*-fused A/B ring junction. Some novel LAH reduction products from **1b**, **2b** and **5** were observed. The antibacterial activities of **1a** and **2a** and other derivatives are also presented.

A CONVENIENT technique for isolating **2a**, and to a lesser extent, **1a**, as crystalline benzene solvates provided ample quantities of these steroid acids from the organic extract of *Cephalosporium acremonium*. At the beginning of our work, the isolation of **1a**[3] was very difficult and when the method for rapid isolation of **2a** was developed, we decided to study its chemistry.

The structures of **2a** and the methyl ester **2b** were established through the mass spectrum of **2b**, which shows a molecular ion *m/e* 546, $C_{32}H_{50}O_7$,† the IR and UV spectra, and the NMR spectrum of **2b**. The latter shows a singlet at δ 1·92 corresponding to the C-16 acetoxy protons of **1b**.[4,5] However, as expected, the second OAc signal at δ 2·06 (s) in the NMR spectrum of **1b** was missing from that of **2b**; therefore, a desacetyl structure is most likely one for **2b**. The carboxyl group of **2a** is part of an α,β-unsaturated acid moiety since the UV spectrum showed an absorption max at 220 mμ (ε 8,000). The absorption due to vicinal OH groups was observed in the IR spectrum of **2b** at 3640 and 3580 cm^{-1}, the latter wave number indicates intramolecular H-bonding between vicinal OH groups.[6]

In addition to these spectral data obtained from **2b**, evidence gathered from some chemical reactions to be subsequently described help establish the structure of **2a**. The presence of vicinal OH groups was further confirmed through the formation of an acetone derivative.[7] The IR spectrum of this acetonide showed a band at 3640 cm^{-1} indicating the presence of still a third OH group. Thus, the seven O atoms of **2a** ($C_{31}H_{48}O_7$) are accounted for as three OH groups, one carboxyl group and one

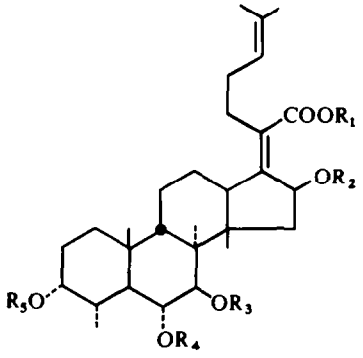
* Present address: Eli Lilly and Company, Indianapolis, Indiana.

† A prominent peak (*m/e* = 486, M-60) indicated the presence of an easily removed OAc group. The M-120 peak prominent in **1b** is a minor peak in **2b**.

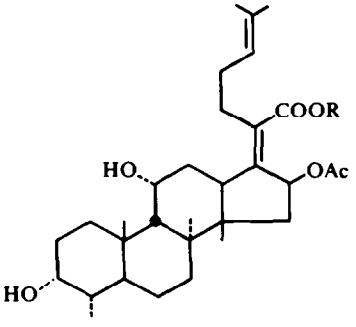
OAc group; this is also consistent with the functional group assignment of **2a**.[4,5] Cephalosporin P_1 (**1a** and **2a**) were later directly isolated from the crude extract in low yield.

Experiments were devised to ascertain placement and configuration of the functional groups in **1a** and **2a**,[8,9] and to test whether all the data obtained were consistent with the recently established *trans–syn–trans* B-boat conformation of fusidic acid (**3a**) type skeleton.[10c]

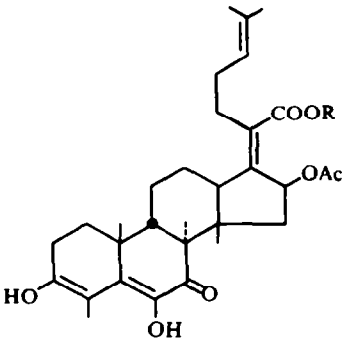
The relative placement of the side-chain carboxyl group and the C-16 OAc group in **1a** was established through lactone formation. When **2a** was pyrolyzed at 240° in nitrogen, the α,β-unsaturated γ-lactone **5**[4] was readily produced in about 35% yield as shown in Scheme I. The structure of **5** was established by elemental analysis and spectral data. The absorptions at 224·5 mμ (ε 13,500) and 1740 cm^{-1} of **5** are character-



	R_1	R_2	R_3	R_4	R_5
1a	H	Ac	H	Ac	H
1b	Me	Ac	H	Ac	H
1c	H	Ac	Ac	H	H
1d	Me	Ac	Ac	H	H
2a	H	Ac	H	H	H
2b	Me	Ac	H	H	H
7	H	H	H	H	H
14	Me	Ac	H	Ac	Ac



3a: R = H
3b: R = Me



12a: R = H
12b: R = Me

istic of an α,β-unsaturated lactone. The mass spectrum of **5** showed a parent ion peak (*m/e* 472, $C_{29}H_{44}O_5$). The M-60 peak prominent in the spectrum of **2b** was no longer present in the spectrum of **5**. Furthermore, the absorption due to the secondary proton attached at C-16 was shifted from δ 5·83 for **2b** to δ 5·05 for **5**.

In order to determine the configuration of the C—O bond at C-16 of **5**, ORD* measurements were made on lactone **5** and the lactone **6**, 3β-acetoxy-16α-hydroxyl-$\Delta^{17(20)}$-bisnor-5αcholenic 22,16-lactone prepared by Mazur[12] (Fig. 1). Since the two

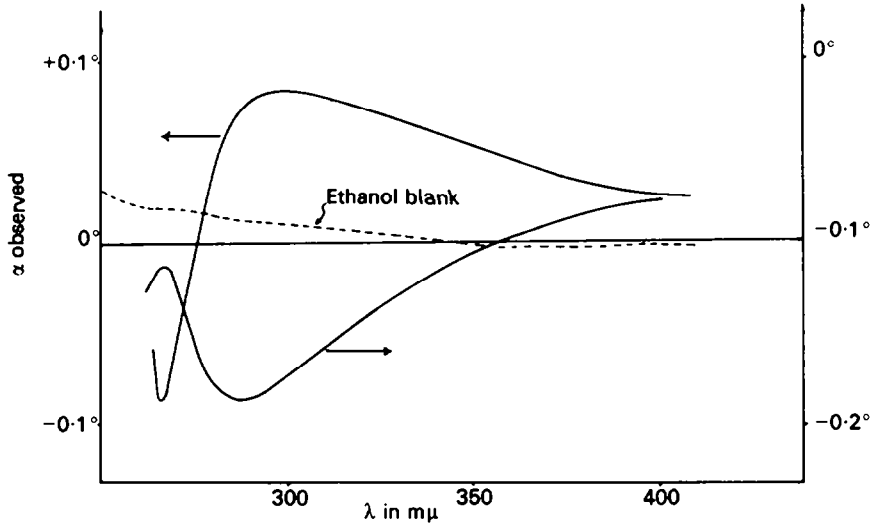


FIG. 1 ORD curves of **5** (upper) and **6** (lower); (*c* 0·150 and 0·165, EtOH, 250 min scan) respectively

lactones had opposite Cotton effects and the lactone **6** has an α configuration at C-16, the β configuration was assigned to C-16 in the lactone **5**. The same configuration at C-16 may be assigned to **1a** and **2a** because **1a** is easily hydrolyzed to the C-16 hydroxy acid **7**, which lactonizes to **5** on acidification.

The structure of the completely hydrolyzed cephalosporin P_1 is suggested as **7** since its NMR, IR and UV spectra show no OAc but OH group absorption. A UV max at 226 mμ (ε 6900) is indicative of an α,β-unsaturated carboxylic acid. Hydrolysis of the C-6 OAc group is apparently assisted by the C-7 OH group since it is hydrolyzed faster than that of the C-16 OAc group.[13] Furthermore, the stepwise alkali hydrolysis of **1a** to **2a** to **7** and finally cyclization to the lactone **5** was accomplished without inversion of configuration† of the functional groups at C-6 or C-16.

To establish whether the alkaline conditions of the benzene solvate extraction procedure could have caused epimerization‡ of **2a** at C-6 and C-16 during isolation,

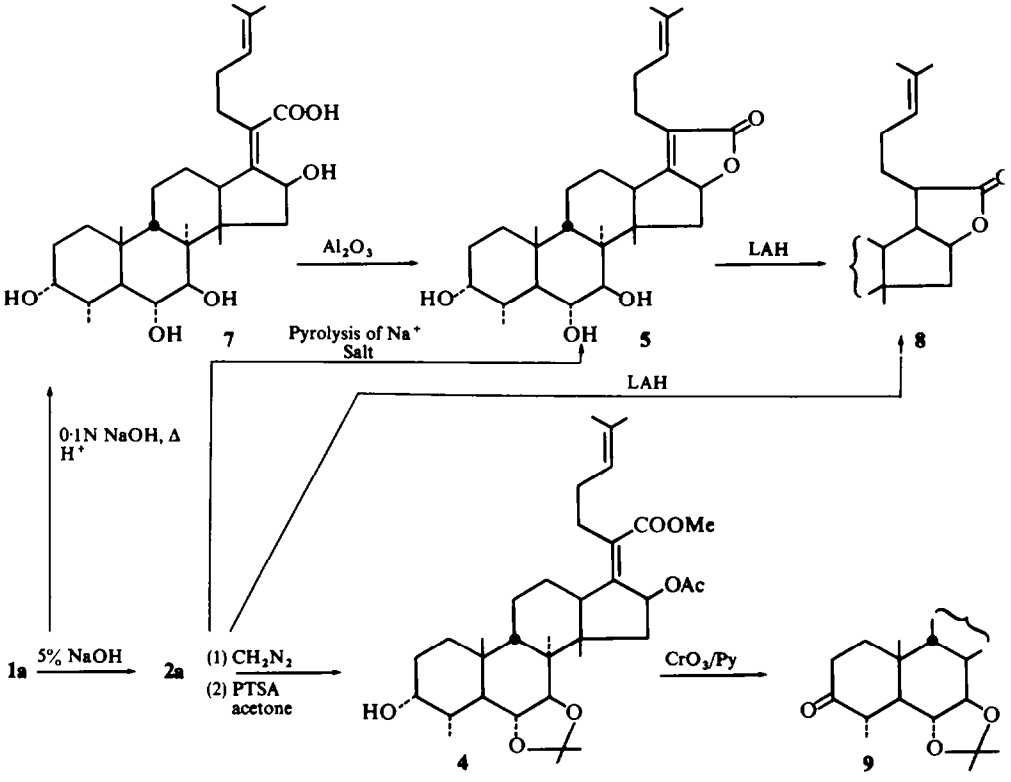
* Arigoni *et al.*[11] showed that the lactone obtained from the 24,25-dihydro derivative of fusidic acid (**3a**) exhibited an intense negative circular dichroism maximum ([θ] 250 = 29,700), which is consistent with the negative Cotton effect of the ORD curve of lactone **5**.

† Sodium hydroxide hydrolysis of the C-16 OAc group of fusidic acid was accomplished without inversion of configuration, but an inversion was reported when sodium bicarbonate was used.[10a]

‡ This point required clarification because acyl transfer and rearrangement through neighbouring group participation during hydrolysis is known, and an inversion in configuration has sometimes been observed.[14]

LAH reductions of both **1b** and **2b** were carried out. The reduction products (each ester gave two) showed identical R_f values. The saturated lactone **8** was a major

SCHEME I.



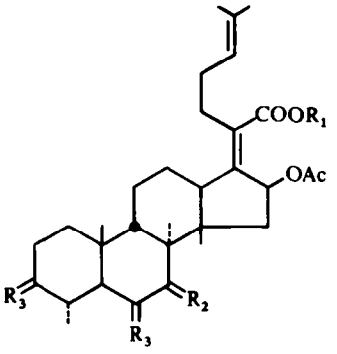
product in each case; the two specimens were shown by X-ray diffraction (XRD) studies to be identical. These results indicate that no epimerization at C-6, C-7, or C-16 had occurred during the isolation of **2a** or the preparation of **7**. The hydride attack of the double bond at position C-17 is assumed to occur from the less hindered α-side to give a *cis*-fused lactone ring. Thus, the saturated γ-lactone **8** probably has the β side-chain configuration[10a] at C-17.

The three OH groups in **2a** were previously placed at C-3, C-6 and C-7.[4] Our spectral and chemical data confirm these assignments. The presence of vicinal OH groups in **2a** and **2b** was shown by the formation of the acetonide **4** from the methyl ester **2b**. IR bands at 1380 and 1385 cm^{-1} and the peak at δ 1·40 (s) in the NMR spectrum are consistent with the acetonide structure. The broad multiplet signal centered at δ 3·70 in the NMR spectrum of **4** is at the same location as the signal arising from the C-3 equatorial proton[5] of fusidic acid. This permits placement of the α-OH group in **4** at C-3.[5, 10b] The structure of **4** is further established by oxidation to **9** with chromium trioxide in pyridine.[15] The NMR spectrum of **9** no longer showed the broad multiplet at δ 3·70 observed for **4**.

The location of the vicinal OH groups in **2a** with respect to the C-3 OH group was established through a series of oxidations described below. Monodesacetylcephalo-

sporin P_1 (**2a**) was first hydrogenated in the presence of 2% Pd–C to give **10a**. Esterification of **10a** with diazomethane gave **10b**. The dihydro derivative **10b** was subjected to chromic acid oxidation[16] which gave the yellow, crystalline triketone **11**. The

1



	R_1	R_2	R_3	R_4	$\Delta^{17,21}$	$\Delta^{24,25}$
10a	H	αH	αOH	αOH	—	2H
		βOH	βH	βH		
10b	Me	αH	αOH	αOH	—	2H
		βOH	βH	βH		
11	Me	=O	=O	=O	—	2H
13a	H	=O	=O	=O	—	—
13b	Me	=O	=O	=O	—	—
15	Me	=O	αOAc	αOAc	—	—
			βH	βH		
16	Me	=O	αOAc	=O	—	—
			βH			
17	Me	=O	2H	=O	—	—
18a	H	αH	αOAc	αOH	—	2H
		βOH	βH	βH	—	
18b	Me	αH	αOAc	αOH	—	2H
		βOH	βH	βH		
19a	H	αH	αOAc	αOH	2H	2H
		βOH	βH	βH		
19b	Me	αH	αOAc	αOH	2H	2H
		βOH	βH	βH		
20a	H	αH	αOH	αOH	2H	2H
		βOH	βH	βH		
20b	Me	αH	αOH	αOH	2H	2H
		βOH	βH	βH		

IR, UV and NMR spectra and elemental analysis substantiate the structure. The UV spectrum of **11** is the most informative. It did not show absorption between 260 and 340 mμ; however, in acidic methanolic solution, two maxima at 219 mμ (ε 8290) and 282 mμ (ε 4500) appeared as shown in Fig. 2. This behavior is strong evidence for the presence of a conjugated triketone system[3b, 17] involving CO functions at C-3, C-6, and C-7 of **1a**; hence the possible placement of the vicinal OH groups at C-11 and C-12 is eliminated. This not only shows the positions of the three OH groups in **2a** at C-3, C-6 and C-7 but also demonstrates that a proton must be present at C-5 to make the conjugated forms **12a** and **12b** possible. Similar triketone derivatives **13a** and

13b were obtained from chromic acid oxidation [16] of **2a** and **2b**, respectively. The UV spectra of **13a** and **13b** in acidic methanol solution in Fig. 2 also resemble that of **11**.

We next sought the relative configurations of the three OH groups at C-3, C-6 and C-7 of **2b**. The ready formation of acetonide **4** from **2b** demonstrates that the OH groups at C-6 and C-7 are vicinal but not necessarily *cis*, since *trans* diol systems are known to form acetone derivatives readily in systems containing a flexible boat conformation.[18] Acetylation was then used to assess the relative reactivity of the three OH groups in **2b**, in the hope of gaining information about their configurations.[19] The reaction mixture texted on silica gel thin-layer plates showed four pronounced spots with R_f values 0·75, 0·68, 0·62, and 0·48.

The spots having R_f 0·75 and 0·68 yielded pure compounds. The latter spot, R_f 0·68, was due to **1b**, and the former spot, R_f 0·75, was found to be the amorphous triacetate **14**, which was also obtained from the acetylation of **1b**. Its structure was established by elemental analysis, and spectroscopic measurements. The IR spectrum of **14** showed the presence of a OH stretching band. The broad multiplet centered at δ 3·70 corresponding to the secondary proton attached at C-3 of **1b** was no longer present in the NMR spectrum, but the singlet at δ 3·50 remained. These data together with the upfield shift of the C-4 Me signal to δ 0·81 (J = 6·0 Hz) indicated that the OH group at C-3 of **1b** was acetylated. The new broad multiplet at δ 4·90 therefore arises from the secondary proton attached to the C-3 carbon holding the newly formed OAc group. The presence of three OAc groups in **14** was also indicated by its mass spectrum. In addition to the parent ion peak m/e 630, prominent peaks corresponding to M-60, M-120 and M-180 were observed.

Several unsuccessful attempts were made to acetylate all the OH groups of **14**. Since one of the two OH groups at C-6 and C-7 in **2a** is very readily acetylated, its orientation is probably equatorial.[19] The C-3 OH group of **2a** reacts next. This behavior is also consistent with the broad multiplet signal and the previous axial assignment[3b] of the C-3 OH. However, the remaining OH group of [2b] is so hindered that it cannot be acetylated under conditions ordinarily used for acetylation of steroids.[7b, 20] Therefore, it must be in a 1,3-diaxial relationship[19] to one of the tertiary Me groups at C-10 or C-14[3b, 10b] and is undoubtedly located at C-6 or C-7.

Structures **1a** and **14** were used to designate tentatively the location and configuration of the functional groups in ring B. The correctness of these structural assignments was established by a series of oxidation and reduction experiments applied to **1b** and spectral studies of the products from these reactions as described below.

The triacetoxy ketone **15** was prepared by chromic acid oxidation of the triacetoxy alcohol **14**.[16] The IR spectrum of **15** showed the absence of OH stretching frequency and the presence of a cyclohexanone absorption at 1710 cm^{-1}. In contrast to the difficult acetylation of the C-7 OH group of **14**, chromic acid oxidation of the C-7 OH group proceeded readily, which supports the axial assignment for this group.[19] Oxidation of **1b** under the same conditions gave the diacetoxy diketone **16**. As expected, the NMR spectrum of **16** showed a downfield shift of the C-4 Me protons signal to δ 1·28 from its initial value of δ 0·90 in **1b**. Other spectral data and the elemental analysis also substantiated structure **16** for the diacetoxy diketone.

When the diacetoxy diketone **16** was subjected to Clemmensen reduction with zinc and acetic acid,[20] the monoacetoxy diketone **17** was formed. The monoacetoxy

diketone **17** was shown by elemental analysis to be contaminated with a small amount of the starting material **16**.

The NMR data for the protons of **1b**, **14**, **15** and **16** at C-3, C-6 and C-7 are shown in Table 1. Since an α and axial proton is known to be present at C-5 in **1b** and the C-8 position is fully substituted,[4, 5] the doublet at δ 4·57 in the spectra of **1b** and **14** is due to the secondary proton at C-6. As expected, the downfield shifts of the doublet signals from δ 4·57 in the spectra of **1b** and **14** to δ 5·30 and δ 5·32 in those of **15** and **16** respectively are due to the influence of a CO group at C-7 of **15** and **16**. The downfield shifts of the signals in the spectra of **15** and **16** corresponding to one of the acetoxy Me protons at δ 2·06 for **1b** and δ 2·04 for **14** to δ 2·18 for **15** and δ 2·20 for **16** are consistent with this observation. The large coupling constants between C-5 and C-6 protons in **1b**, **14**, **15**, and **16** indicate that these are diaxially coupled; therefore, the C-6 proton must be β and axial. Thus, the C-6 OAc in **1b** and in its derivative, **14**, **15**, and **16** must be α and equatorial. A distortion in boat conformation in ring B of **15** and **16** is evident since the coupling constant between the C-5 and C-6 protons changed from 10 Hz for **1b** and **14** to 13 Hz for **15** and **16**. Additional evidence that the stereochemical assignment of the 6 α-OAc group for **1a**, **14**, **15**, and **16** is correct was obtained from the ORD data of **15**, **16**, and **17**, which showed negative Cotton effects with successive decrease in amplitude in their ORD curves from $[\alpha]_{317}$ −1025° for **15** to $[\alpha]_{320}$ −510 for **16** and $[\alpha]_{320}$ −398 for **17**.

Examination of Dreiding models employing a ring B-boat conformation for **15**, **16**, and **17** and application of the octant rule[21] shows the 6 α-OAc group is in a negative octant relative to the CO at C-7, and thus a substantial negative contribution can be expected. A positive contribution to the Cotton effect could be expected from a 3-keto group appearing in a positive octant; this is in keeping with the change in negative value from −1025° for **15** to −516° for **16**. The removal of the 6 α-OAc group would be expected to cause the observed change in negative value from −516° for **16** to −398° for **17**.

As previously mentioned during the discussion of the acetylation reactions of **2b**, one of the vicinal OH groups in ring B was probably 1,3-diaxial in its relationship to one of the tertiary Me groups at C-10 or C-14. Since the equatorial OAc group is now located at C-6, the OH group is at the C-7 position and is 1,3-diaxial to the Me group at C-14. Additional evidence for assigning axial configuration of the C-7 OH group was provided by the NMR spectra of compounds **1b** and **15** (Table 1). Because of the anisotropic effect of the C-7 CO group in **15** in the signal due to the C-32 Me attached to C-8 was shifted downfield to δ 1·34 from δ 1·18 of **1b** ($\Delta\delta = 0{\cdot}16$), and the C-19 Me (attached at C-10) to δ 1·18 from δ 1·05 of **1b** ($\Delta\delta = 0{\cdot}13$), but the C-18 Me (attached to C-14)[10b] signal was shifted upfield to δ 0·92 from δ 1·18 of **1b**, with an upfield shift of δ 0·26. This large upfield shift occurs because the C-18 Me group in **1b** was shielded by the C-7 OH group,[11, 22] which was at 1,3-diaxial position with it. Should the C-7 OH group in **1b** be α and equatorial, the interaction between C-7 OH and C-18 Me would not be so large[22] and could cause upfield change of only about δ 0·15.[11]

Another piece of evidence was provided by measuring the NMR spectra of **1b** and **3b** in deuteriochloroform and in deuteriopyridine[23] as shown in Table 2. The large downfield shift ($\Delta\delta = 0{\cdot}41$) of C-32 Me in **1b** indicated that C-7 OH group which situated next to the angular Me was axial and *trans*[24] to the Me group. Further-

TABLE 1. NMR DATA FOR FUSIDIC ACID AND CEPHALOSPORIN P_1 DERIVATIVES[a]

	1b	1d	2b	3b	14	15	16
Me at C-4	0·92 (d) J = 6·5 Hz	0·93 (d) J = 6·5 Hz	0·93 (d) J = 6·0 Hz	0·92 (d) J = 6·5 Hz	0·81 (d) J = 6·0 Hz	0·81 (d) J = 6·5 Hz	1·28 (d) J = 6·5 Hz
Me at C-8	1·18 (s)	1·20 (s)	1·25 (s)	1·37 (s)	1·18 (s)	1·34 (s)	1·36 (s)
at C-10	1·18 (s)	1·07 (s)	1·15 (s)	0·98 (s)	1·18 (s)	1·18 (s)	1·18 (s)
or C-14	1·05 (s)	1·00 (s)	1·00 (s)	0·91 (s)	1·06 (s)	0·92 (s)	0·92 (s)
Me at C-26, -27	1·60 (d) J = 1 Hz	1·60 (d) J = 1 Hz	1·60 (d) J = 1 Hz	1·60 (d) J = 1 Hz	1·60 (d) J = 1 Hz	1·60 (d) J = 1 Hz	1·61 (d) J = 1 Hz
MeCOO	1·68 (d) J = 1 Hz	1·68 (d) J = 1 Hz	1·68 (d) J = 1 Hz	1·68 (d) J = 1 Hz	1·68 (d) J = 1 Hz	1·67 (d) J = 1 Hz	1·69 (d) J = 1 Hz
at C-3	—	—	—	—	2·04 (s)	2·04 (s)	—
at C-6	2·06 (s)	—	—	—	2·04 (s)	2·18 (s)	2·20 (s)
at C-7	—	2·06 (s)	—	—	—	—	—
at C-16	1·96 (s)	1·94 (s)	1·92 (s)	1·98 (s)	1·98 (s)	1·97 (s)	1·97 (s)
2°H at C-3	3·70 (bm)	3·70 (bm)	3·67 (bm)	3·75 (bm)	4·90 (bm)	4·90 (bm)	—
2°H at C-6	4·57 (d) J = 10 Hz	3·42 (d) J = 10 Hz	3·40 (d) —	— —	4·58 (d) J = 10 Hz	5·30 (d) J = 13 Hz	5·32 (d) J = 13 Hz
2°H at C-7	3·50 (s)	4·65 (s)	3·40 (s)	—	3·50 (s)	—	—
2°H at C-11	—	—	—	4·36 (m)	—	—	—
2°H at C-16	5·87 (d) J = 8·5 Hz	5·83 (d) J = 8·5 Hz	5·83 (d) J = 8·5 Hz	5·86 (d) J = 8·5 Hz	5·85 (d) J = 8·5 Hz	5·82 (d) J = 8·5 Hz	5·85 (d) J = 8·5 Hz
Vinylic H at C-24	5·13 (bt)	5·12 (bt)	5·13 (bt)	5·12 (bt)	5·12 (bt)	5·13 (bt)	5·13 (bt)
COOMe at C-21	3·63 (s)	3·62 (s)	3·62 (s)	3·63 (s)	3·68 (s)	3·62 (s)	3·65 (s)

[a] NMR spectra were recorded on a Varian HR-60 instrument in $CDCl_3$ solns and calibrated against tetramethylsilane. Chemical shifts are given in δ values.

TABLE 2. COMPARISON OF PROTON MAGNETIC RESONANCE OF METHYL GROUPS FOR **1b** AND **3b** IN CHLOROFORM AND PYRIDINE

	1b		**3b**	
	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N
Me at C-4	0·92 (d) J = 6·5 Hz	1·18 (d) J = 6·5 Hz	0·92 (d) J = 6·5 Hz	1·15 J = 6·5 Hz
Me at C-8	1·18 (s)	1·59 (s)	1·37 (s)	1·64 (s)
Me at C-10	1·05 (s)	1·21 (s)	0·90 (s)	1·10 (s)
Me at C-14	1·18 (s)	1·45 (s)	0·97 (s)	1·10 (s)

more, the C-32 Me signal in **3b** was shifted from δ 1·37 in chloroform to δ 1·64 in pyridine, a shift of δ 0·27. The same phenomenon was found in **1b**. The C-18 Me resonance was shifted from δ 1·18 in chloroform to δ 1·45 in pyridine, a shift of also δ 0·27. Examination of Dreiding models revealed that the relationship between the C-11 OH and the C-32 Me in **3b** is similar to the relationship of the C-7 OH and the C-18 Me in **1b**, provided that the C-7 OH is axial. In view of the above NMR data, the C-7 OH group in **1b** is axial in configuration.

The side-chain structure previously proposed for cephalosporin P_1 is also in agreement with that for **3a**.[10a] An identical side-chain structure is also derived from the di- and tetrahydro derivatives of **1a** and **2a**.

The easily reduced double bond ($\Delta^{24,25}$) in **1b** and **2b** was shown to be trisubstituted through observation of the vinylic proton signal at δ 5·13 (bm) as shown in Table 1. This signal was no longer observed in the spectra of the dihydro derivatives **10b** and **18b**. The double bond ($\Delta^{24,25}$) is part of an isopropylidene moiety since acetone was obtained on ozonization of **2b**. The tetrasubstituted double bond of **1a** and **2a** was shown to be $\Delta^{17,20}$ since absorption at 220 mμ was observed.

The antibacterial activities of **3a*** and the derivatives prepared from **1a** and **2a** are compiled in Table 3. Cephalosporin P_1 (**1a**) is shown to be the most active antibiotic among the derivatives of **1a** and **2a**. It is also of interest that derivatives of **2a** are consistently less active than the corresponding derivatives of cephalosporin P_1.

Although no direct chemical correlation between **1a** and **3a** has been reported, the NMR and mass spectral data previously compiled[4,5] and the data reported in Table 1 strongly support a common carbon skeleton.[10b,c] The C-3 OH group in ring A of **1a** was previously shown to be α and axial and the C-4 Me group α and equatorial.[3b] These conclusions are in accord with the configuration of C-3 OH group and C-4 Me group **3a**. Thus, the structures for cephalosporin P_1 (**1a**) and its monodesacetyl derivative **2a** are established as shown.

Following the determination of structures of **1a** and **2a**, attention was shifted to the isolation of new components from the crude organic extract of *Cephalosporium acremonium*. One new component was isolated through preparative TLC on silica gel PF. A sample of the methyl ester derivative of this new component was obtained which gave a single spot on a TLC plate, although it was not obtained in crystalline form. The new component was shown to have structure **1d** from the spectral studies

* Ramycin isolated from *Mucor ramannianus* was shown (R.T.R.) to have an identical mass spectrum with that of **3a** and their antibacterial activity against *S. aureus* 3055, *S. lutea*, *C. diphtheriae* X-166, *B. subtilis* X-12 and *M. tuberculosis* were entirely the same.[25]

and elemental analysis, which gave a molecular formula of $C_{34}H_{52}O_8$. Its NMR spectrum showed signals corresponding to two OAc protons, and its mass spectrum showed a peak at *m/e* 528, which apparently is the M-60 peak instead of parent ion peak. The NMR spectrum resembled that of **1b** in general features. However, there are minor differences since a singlet appeared at δ 4·65 and a doublet at δ 3·42 (J = 10 Hz) instead of a singlet at δ 3·50 and a doublet at δ 4·57 for **1a**. This new component is best described by adopting Oxley's isocephalosporin P_1 structure **1c**.[9] Comparing the IR and NMR spectra of **1d** with Oxley's isocephalosporin P_1 methyl ester showed that these were identical.*

Since such a diol-monoacetate system was known to isomerize readily,[13] several experiments were carried out in the hope that **1b** could be isomerized to this new component **1d**.[9]

Cephalosporin P_1 (**1a**) was found to be isomerized to **1c** after stirring **1a** with 5% sodium carbonate solution in water for 5 hr. After work-up and esterification, two spots at R_f 0·62 and 0·44 were detected on TLC. The spot of R_f 0·62 is the same as the starting material **1b** and the spot of R_f 0·44 proved to be the same as that of the new component **1d** by the TLC and elemental analysis, as well as NMR and mass spectroscopy studies. Whether its corresponding acid **1c** was originally present in the crude organic extract of *Cephalosporium acremonium* or formed during the isolation process remains unknown.

EXPERIMENTAL

M.ps were taken in capillary m.p. tubes using a Thomas-Hoover apparatus and are uncorrected. The elemental analyses, ORD and X-ray diffraction measurements were carried out in the Analytical Laboratories of Eli Lilly and Company. The major portion of the NMR spectral determinations using a Varian Association HR-60 spectrometer were also obtained from Eli Lilly and Company. Other instrumental determinations involving use of a Cary 14, Beckmann IR-5A, and Varian A-60 spectrometers were made at Oklahoma State University. A LKB mass spectrometer-gas chromatograph was also used for mass spectrometry.

Isolation of cephalosporin P_1 (**1a**) *and monodesacetylcephalosporin P_1* (**2a**). The most frequently used isolation procedure was a benzene solvate technique which afforded **1a** or **2a** from the crude butyl acetate extract. Column chromatography using silica gel was also used but found to be less effective and more troublesome.

A. *Isolation of* **2a** *through a benzene solvate procedure.*[10a] The concentrate of crude organic extract (3 g) of *Cephalosporium acremonium* was dissolved in 50 ml n-butyl acetate, filtered and stirred at room temp with 40 ml 5% NaOH aq. The aqueous layer was separated and 50 ml benzene were added. The pH was adjusted to 6 by adding 10% HCl aq. The benzene layer was separated, allowed to stand overnight, and benzene solvate crystals were filtered out. The solvate was washed with water and with benzene several times until the washings were colorless. The crystals of benzene solvate were then dissolved in MeOH and evaporated to near dryness with a vacuum evaporator to give a colorless solid which was recrystallized twice from aqueous MeOH and once from isopropyl alcohol to yield 540 mg (18% yield) of colorless solid **2a**: m.p. 197–198·5°; $[\alpha]_D$ +37·6° (*c* 0·5, MeOH); IR (KBr) 3400, 2920, 1700, 1440, 1370 and 1260 cm^{-1}; UV max (CH_3OH) 220 mμ (ε 8,000).

TLC on silica gel showed one spot, R_f 0·58 (solvent system: pet ether–acetone–EtOH = 4:10:1, thin layer thickness 0·35 mm). A sample of crystalline **2a** was assayed by paper chromatography using 70% propanol as eluting solvent. It was found to be active against a sensitive *Staph. aureus* 3055 as shown in Table 3. A mass spectrum was obtained but the molecular ion did not appear. (Found: C, 69·46; H, 9·26. $C_{31}H_{48}O_7$ requires: C, 69·89: H, 9·08%).

B. *Isolation of* **1a** *through a benzene solvate procedure.* A procedure identical to part A was used except that water was substituted for 5% NaOH aq. This technique yielded 80 mg of benzene solvate which finally gave 40 mg (1·4%) of **1a**: m.p. 147–148°; IR ($CHCl_3$) 2920, 2800–2550, 1720, 1460 and 1375 cm^{-1}.

* We thank Dr. P. Oxley for samples of isocephalosporin and its methyl ester.

TABLE 3. ANTIBACTERIAL ACTIVITY[a]

	Coryne-bacterium diphtheriae	*Sarcina lutea*	*Sarcina subflava*	*Staph. aureus* 3055	*Staph. aureus X-1*	*C. diphtheriae X-166*	*B. subtilis X-12*	*M. tubercu-losis*
1a	0·05	3·13	0·78	0·39	1·56	—	—	—
2a	0·39	12·5	12·5	50	100	0·4	100	100
3a	—	0·8	—	0·4	—	0·0125	1·6	25
5	6·25	25	6·25	100	100	—	—	—
7	12·5	25	25	100	100	—	—	—
10a	0·39	6·25	12·5	6·25	25	—	—	—
11	6·25	12·5	100	50	100	—	—	—
16	6·25	100	100	50	100	—	—	—
18a	0·10	6·25	3·13	0·39	0·78	—	—	—
20a	6·25	100	100	100	100	—	—	—

[a] Minimum inhibitory concentration (mcg/ml).

C. *Isolation of* **1a** *and* **2b** *and* **1d** *by preparative TLC.* The crude organic extract of *Cephalosporium acremonium* (3 g) was dissolved in 2 ml acetone and separated on a column packed with 50 g Davidson silica gel (grade 62, mesh 60–200). The eluant (1 l.) was gradually changed in 50 ml cuts from n-hexane to 30% acetone in n-hexane. These fractions were concentrated in a vacuum evaporator and then tested on a thin-layer plate coated with 0·30 mm thickness of silica gel (Silica Gel $PF_{254+366}$, Brinkmann Instruments, Inc). Three fractions eluted with 25% acetone in n-hexane were found to be enriched in **1a**. These fractions were combined and eluted twice with 20% acetone in n-hexane (500 ml), decolorized by eluting through a column packed with 5 g of active carbon. The colorless eluate was evaporated to dryness and crystallized in MeOH to give 100 mg of **1a**, m.p. 147–148°. The identity of **1a** was established by conversion to **1b**.

A 1 g sample of esterified crude cephalosporin P was eluted through a column packed with 25 g of Davidson silica gel in the same way as described for chromatographic isolation of **1a**. The fractions (25–30% acetone in n-hexane) were found to be enriched with **2b** and **1d**. These fractions were combined and concentrated to give about 80 mg of yellow residue. The residue was dissolved in 1 ml of MeOH and applied to glass plates coated with 0·80 mm of silica gel. After developing in a chamber containing 1·5% MeOH in $CHCl_3$, the spots were detected with an UV lamp (254 mμ). Two intense spots were scraped from the plates, and the silica gel was extracted with MeOH and filtered. The more polar spot R_f 0·21) gave 27 mg crystals, m.p. 224–228°. These crystals were identified as **2b** through X-ray diffraction comparison with an authentic sample. The less polar spot gave 32 mg of **1d** (R_f, 0·44) as non-crystalline material, IR (KBr) 3500–3170, 2910, 1725, 1460, 1380, and 1255 cm^{-1}; UV max ($CHCl_3$) 220 mμ (ε 8500). The NMR data for **1d** are shown in Table 1. The mass spectrum for **1d** showed the following peaks at *m/e* 528 (M-60), 468 (M-120), 519 (M-69), 496 (M-60-32), 459 (M-60-69), 450 (M-120-18), 436 (M-120-32), 427 (M-60-69-32), 399 (M-120-69), 381 (M-120-69-18), 367 (M-120-69-32), 363 (M-120-69-36), and 349 (M-120-69-32-18).

Isolation of cephalosporin P_1 (**1a**) *as* **1b**. A soln of 3 g of the organic extract of *Cephalosporium acremonium* in 150 ml ether was esterified with diazomethane as described. The crude cephalosporin P methyl ester was evaporated to dryness, dissolved in 2 ml acetone and eluted through a column packed with 50 g Davidson silica gel (grade 62, mesh 60–200) as described for chromatographic isolation of **1a**. A sample of **1b** (600 mg) thus obtained melted at 196–197°.

Sodium carbonate-catalyzed isomerization of **1a** *to* **1c**. A 50 mg sample of **1a**, m.p. 147–158°, was dissolved in 10 ml Na_2CO_3aq and stirred at room temp for 5 hr. At that time, an equilibrium concentration of **1a** and **1c** was reached[9] as shown by TLC. Attempt to separate **1a** and **1c** on thin-layer plates was unsuccessful. However, when the mixture of **1a** and **1c** was esterified by diazomethane, they were readily separated in a solvent system of 2% MeOH–$CHCl_3$ on silica gel plate. Two spots (R_f 0·60 and 0·42) were detected with I_2. These corresponded to **1b** and **1d** respectively. The identity of the spot having R_f 0·42 with **1d** was made through preparative TLC and IR comparisons of the sample isolated from the TLC plate.

Preparation of cephalosporin P_1 *methyl ester* (**1b**) *from* **1a**. To a soln of 300 mg of **1a**, m.p. 147–148°, in 200 ml ether was added an ethereal soln of freshly prepared diazomethane until a yellow color persisted for 30 min. Dil AcOH was then added to destroy the excess diazomethane. The ether soln was washed with water, dil $NaHCO_3$aq and again with water. The ether layer was evaporated to dryness in a vacuum evaporator and the residue was crystallized in ether. Recrystallization in MeOH gave crystals of **1b**: m.p. 196–197°; $[\alpha]_D^{25°}$ +28° (*c* 1·2, $CHCl_3$); UV max (MeOH) 220 mμ (*e* 11,000); IR ($CHCl_3$) 3500–3200, 2920, 2850, 1720, 1460, 1375, and 1255 cm^{-1}. The NMR data for **1b** are shown in Table 1. The X-ray diffraction pattern of **1b** from this route and an authentic sample were found to be identical.* (Found: C, 69·18; H, 8·72. $C_{34}H_{52}O_8$ requires: C, 69·36; H, 8·90%).

Preparation of **2b** *from* **2a**. A 0·5 g sample of **2a**, m.p. 197–198·5°, was esterified with diazomethane as described. The resulting **2b** was crystallized in ether–hexane and from MeOH to give 0·48 g of **2b**, m.p. 232–233·5°; $[\alpha]_D^{25°}$ +29° (*c* 0·5, MeOH); UV max (EtOH) 220 mμ (ε 8375); IR ($CHCl_3$) 3640 (monomer OH), 3580 (dimer OH), 3500–3200 (intermolecular H-bonding OH), 3080, 2980, 2950, 1740 (acetate CO), 1718 (α, β-unsaturated ester CO) 1433, 1373, 1255 (ester), 1015, and 785 cm^{-1}. The mass spectrum showed a parent ion peak *m/e* 546 ($C_{32}H_{50}O_7$). The NMR data are given in Table 1. This sample showed no depression in m.p. on mixing with authentic **2b**. Additional comparisons were made through IR spectroscopy and TLC. (Found: C, 70·54; H, 9·22. $C_{32}H_{50}O_7$ requires: C, 70·30; H, 9·22%).

Preparation of the methyl fusidate (**3b**).[10a] A 100 mg sample of sodium fusidate was dissolved in 5 ml of water and the soln was acidified by dropwise addition of 0·1N HCl. The resulting ppt was extracted with ether and esterified with diazomethane in the same manner as described for **2a**. Methyl fusidate (**3b**)

* We are grateful to Dr. T. G. Halsall for a sample of cephalosporin P_1.

crystallized out when the ethereal soln was concentrated through evaporation. Recrystallization in MeOH gave 96 mg of **3b**, m.p. 153–154°. The NMR data of **3b** are shown in Table 1.

Ozonolysis of **2b**. Ozononized O_2 was bubbled through a soln of 25 ml dry CH_2Cl_2 and 0·2 ml dry pyridine containing 100 mg **2b** at dry ice temp for 10 min until the color of the CH_2Cl_2 soln turned blue. Zn dust (1·25 g) and 2·5 ml AcOH were then added. After stirring at 0° for 20 min, the ppt was filtered off and washed with CH_2Cl_2. The CH_2Cl_2 soln was steam distilled into a soln of 20 ml water and 3 ml conc H_2SO_4 containing 80 mg 2,4-dinitrophenylhydrazine. The distillate containing the precipitated 2,4-dinitrophenylhydrazone was separated into 2 layers (lower CH_2Cl_2 layer and lighter aqueous H_2SO_4 layer). The 2 layers were separated and the aqueous H_2SO_4 layer was extracted with three 10 ml portions CH_2Cl_2. These extracts were combined and evaporated to near dryness. Yellow crystals (m.p. 114–118°) formed after standing for a short time. Recrystallization from aqueous EtOH raised the m.p. to 120–130°. There was no depression in m.p. on mixing with acetone 2,4-dinitrophenylhydrazone. The IR spectrum and the R_f, 0·62, of the isolated acetone–dinitrophenylhydrazone were identical with those of an authentic sample.

Pyrolysis of **2a** *to the lactone* **5**. A 175 mg sample of **2a** was placed in a 10 ml round-bottomed flask and pyrolyzed at 240° in a N_2 atm in a salt bath (8·5 g of KNO_2 + 10 g of KNO_3) for 5 min, when the evolution of gases almost ceased. The yellow amorphous product weighing 133 mg was spotted on a glass plate coated with a thin layer of alumina and then developed in a 1:3 pet ether and acetone solvent system. Four spots having R_f 0·58 (trace), 0·47 (major, grey), 0·27 (green) and 0·04 (grey-brown) as well as a stationary spot corresponding to **2a** were observed after spraying with H_2SO_4. Column chromatography of 124 mg of the yellow amorphous solid on an alumina column (20 g alumina, grade V deactivated with 15% water) gave white needle-like crystals: m.p. 180–181°; $[\alpha]_D^{25°}$ +76·2° (*c* 0·95, EtOH); UV max (EtOH) 224·5 mμ (ε 13,500); IR ($CHCl_3$) 3460, 3050, 2910, 1740 (α,β-unsaturated γ-lactone), 1460, 1380 cm^{-1}. The NMR spectrum showed that lactone **5** no longer contained an OAc group (Table 1). The mass spectrum showed a parent ion peak *m/e* 472. The ORD curve of the lactone **5** showed $[\alpha]_{400}$ +20°, $[\alpha]_{295}$ +57°, $[\alpha]_{265}$ −27°, $[\alpha]_{261}$ −14° (*c* 0·15, 1 cm, EtOH). This curve is a mirror image of the ORD curve obtained for **11**, which showed $[\alpha]_{400}$ −45°, $[\alpha]_{287}$ −116°, $[\alpha]_{267}$ −67°, and $[\alpha]_{263}$ −76° (*c* 0·165, 1 cm, EtOH).[12] (Found: C, 73·58; H, 9·42. $C_{29}H_{44}O_5$ requires: C, 73·69; H, 9·38%).

Alkaline hydrolysis of **2a** *to* **5**. A 90 mg sample of **2a** was dissolved in a minimum amount of dry acetone (2 ml) and 1 ml MeOH. The pH was adjusted to 7·5–8·0 with a 33% NaOH aq and the soln was then poured into 50 ml acetone. The Na salt of **2a** was separated by filtering through a sintered-glass funnel and was then washed with dry acetone, dissolved in water and acidified by addition of 0·1N HCl which gave a white ppt. This was dissolved in ether and the ether soln was washed with water, dil $NaHCO_3$ aq and water and then dried over Na_2CO_3. Chromatography on a Florisil column gave 48 mg of a white crystalline **5**: m.p. 179–180° +75·5° (*c* 0·83, EtOH). There was no depression in m.p. on admixture with **5** prepared by pyrolysis of **2a** and only one spot was observed on the silica gel TLC of a mixture of **5** from the two sources The IR, UV, NMR and ORD spectra of **5** from the two sources were identical. (Found for this sample of **5**: C, 73·59; H, 9·42. $C_{29}H_{44}O_5$ requires: C, 73·69; H, 9·38%).

Alkaline hydrolysis of **1a** *and* **2a** *to* **7**. A 100 mg sample of **2a**, m.p. 197–198·5°, obtained from 5% NaOH aq hydrolysis of **1a** via benzene solvate procedure was added to 3·8 ml 0·1N HCl and the mixture was heated at reflux for 4 hr. The resulting ppt was filtered off, washed with water and crystallized and then recrystallized from MeOH and water to yield 79 mg 125–126·5°; IR (KBr) 3400, 2800, 1710 and 1700 (α,β-unsaturated carboxylic acid) cm^{-1}, the acetate band at 1260 cm^{-1} was missing; UV max (EtOH) 226 mμ (ε 6900). Addition of alkali caused a shift in absorption max to longer wavelength. The NMR spectrum no longer showed the peak due to acetate Me protons. In an attempt to purify **7** on a silica gel column (Davidson, grade 62, mesh 60–200), compound **5**, m.p. 181°, was obtained in 90% yield. It was identified through the m.p. of an admixture which showed no depression and IR, UV, NMR, mass spectra and X-ray diffraction comparisons. (Found: C, 70·01; H, 9·33. $C_{31}H_{48}O_7$ requires: C, 69·89; H, 9·08%).

Lithium aluminium hydride reduction of **1b**, **2b**, *and* **5**. LAH (1 g) was heated in 100 ml refluxing glyme with good stirring for 10 min. Heating was stopped and a soln of 100 mg of **2b** in 100 ml glyme was added quickly. After the exothermic reaction had slowed, heating was resumed to maintain reflux for about 30 min and the reaction mixture was then allowed to cool. A Na_2SO_4 aq was added gradually to the stirred suspension until a white ppt formed and settled to the bottom of the flask. The suspension was filtered off and the filtrate was dried to give 27 mg solid, which was shown to contain two components having R_f 0·35 and 0·61.

The same procedure was used to reduce **1b** and **5**, and the resulting solid also contained the two components with R_f 0·35 and 0·61. The major component **8** (R_f 0·61) was obtained in crystalline form, m.p.

222–223°. The reduction product **8** from the 3 sources was shown to be identical by X-ray diffraction studies. The IR spectrum of **8** showed a band at 1770 cm^{-1} indicative of a saturated γ-lactone. The mass spectrum of **8** showed peaks at *m/e* 474 and *m/e* 392, which represent the parent ion of $C_{29}H_{46}O_5$ and side-chain elimination with H-transfer through an expected McLafferty rearrangement. The mass spectrum of the unsaturated lactone **5**, does not show a peak corresponding to the elimination of the side-chain. The UV spectrum of **8** did not show absorption in the region of 225 mμ and the ORD curve did not have a Cotton effect in the vicinity of 266 mμ.[26]

The material with R_f 0·35 was readily converted to **8**, R_f 0·61, by obtaining a pure sample from a preparative thin-layer separation and treating it with MeOH containing HCl.

Preparation of the acetonide **4** *from* **2b**. A 100 mg sample of **2b**, m.p. 232–233·5°, was dissolved in 2 ml dry acetone, and to this soln were added 20 mg of *p*-toluenesulfonic acid (monohydrate) and a few drops of 2,2-dimethoxypropane to react with any water. The reaction mixture was brought to pH 7–8 with bicarbonate soln, 2 ml water were added, and the soln was evaporated under reduced press. The resulting ppt was dissolved in ether and the ether soln was washed with water and bicarbonate soln and then dried and filtered. A similar ether soln was obtained from the aqueous portion of the reaction mixture. These ether solns of crude acetonide were spotted on a silica gel thin-layer plate, developed with a mixture of EtOAc, $CHCl_3$ and MeOH (5:5:2). Two spots were observed. These are due to **2b**, R_f 0·53, and acetonide **4**, R_f 0·64. The ether solns were combined and evaporated to give 90 mg solid which was eluted with pet ether from a 1 × 15 cm column of basic alumina to give 30 mg of **4**: m.p. 158–159·5°; UV max (C_2H_5OH) 220 mμ (ε 5650); IR (KBr) 3640, 3020, 2900, 2800, 1740, 1730, 1465, 1385, 1380, 1236 and 1175 cm^{-1}; NMR ($CDCl_3$) δ 1·40 (s, 6, *gem*-dimethyl). Some starting material, **2b**, (6 mg) was eluted with ether–MeOH. (Found: C, 71·65; H, 9·25. $C_{35}H_{54}O_7$ requires, C, 71·64; H, 9·28%).

Oxidation of acetonide **4** *to* **9**. The acetonide **4** (20 mg) in 0·3 ml pyridine was added to a slurry of CrO_3 pyridine complex[15] containing 6·2 × 10^{-4} moles CrO_3. The product was extracted with ether, the extract was dried ($MgSO_4$) concentrated and the product was purified by eluting through a basic alumina column. The amorphous solid **9** (8 mg) eluted in the 5% acetone in $CHCl_3$ fraction did not crystallize but gave a single spot on a silica gel thin-layer plate; IR (KBr) 2900, 1740, 1710, 1465, 1380, 1368, and 1240 cm^{-1}.

Hydrogenation of **2a** *and* **2b** *to* **10a** *and* **10b**. A soln of **2a** (100 mg, m.p. 197–198·5°) in 25 ml abs EtOH hydrogenated at room temp using 20 mg of 2% Pd/C catalyst. After 30 min, the catalyst was filtered out and water was added to the filtrate. The resulting ppt crystallized from MeOH–water yielded 91 mg of **10a**: m.p. 201–202°; UV max (CH_3OH) 222 mμ (ε8000). (Found for **10a**: C, 69·59; H, 9·39. $C_{31}H_{52}O_7$ requires: C, 69·63; H, 9·43%).

The hydrogenation of **2b** prepared by diazomethane esterification of **2a** was carried out in the same manner to give a 90% yield of **10b** which after two crystallizations from MeOH melted at 262–263°: UV max (MeOH) 220 mμ (ε 8375); mass spectrum *m/e* 548 (molecular ion). The NMR spectrum (Table 1) no longer showed the peak due to the vinylic proton at C-24 of **2b**.[5] (Found for **10b**: C, 69·71; H, 9·55. $C_{32}H_{52}O_7$ requires: C, 70·04; H, 9·55%).

Ozonization of **10b**. A 30 mg sample of **10b** in 20 ml CH_2Cl_2 was treated in the same manner as described for the ozonization of **1b**. No acetone dinitrophenylhydrazone was obtained in this case.

Preparation of the 3,6,7-*triketone* **11**. A 63 mg sample of **15b** was dissolved in 2 ml AcOH and 0·3 ml of chromic acid soln (10% of CrO_3 in 95% AcOH) was added dropwise with vigorous shaking until an orange tint persisted. The reaction mixture then was poured into 50 ml water and the resulting ppt was extracted with ether. The ether soln was washed with water and dil $NaHCO_3$ aq and water and then dried ($MgSO_4$) and concentrated under reduced press to near dryness. Crystallization from EtOH–water gave 10 mg yellow crystals, m.p. 193–198°. After two crystallizations from EtOH–water the m.p. rose to 205–208°; UV max (MeOH, H^+) 219 and 282 mμ (ε 8290 and 4500), 260–340 mμ transparent. Similar absorptions were also observed in alkaline soln as shown in Fig. 2. (Found: C, 68·08; H, 8·33. $C_{32}H_{46}O_7$ requires: C, 68·54; H, 8·63%).

Preparation of the 3,6,7-*triketone* **13a**. A soln of 40 mg of **2a** in glacial AcOH was treated with 0·5 ml soln of 5% CrO_3 in 95% AcOH in the same way as **15b** to give 8 mg yellow crystals of **13a**, m.p. 172–177°. The UV spectrum in MeOH and acidified MeOH was the same as that of **11**. (Found: C, 67·96; H, 8·36. $C_{31}H_{42}O_7 \cdot H_2O$ requires: C, 68·34; H, 8·14%).

Preparation of the 3,6,7-*triketone* **13b**. A soln of 50 mg of **2b** in glacial AcOH was treated with 0·6 ml 5% CrO_3 in 95% AcOH as before to give 15 mg yellow crystals of **13b**: m.p. 207–210°; UV transparent 260–340 mμ, UV max (MeOH, H^+) 219 and 283 mμ as shown in Fig. 2. (Found: C, 69·19; H, 8·38. $C_{32}H_{44}O_7 \cdot H_2O$ requires: C, 68·79; H, 8·30%).

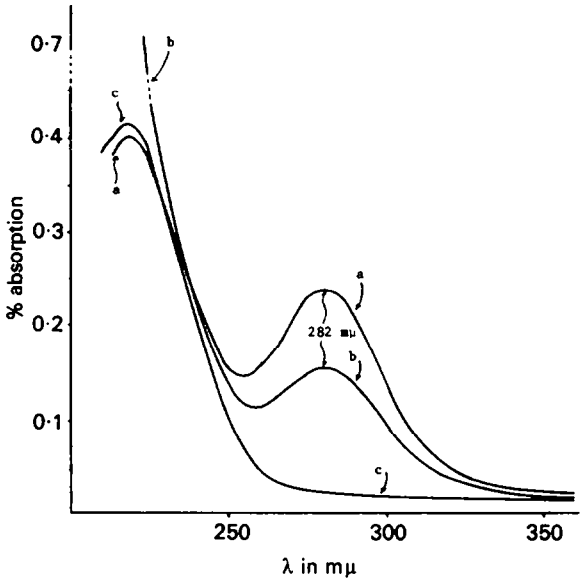


FIG. 2 UV curves of **11**, **13a** and **13b** in (a) MeOH(H^+), (b) MeOH(OH^-), and (c) MeOH.

Preparation of methyl 3-acetoxycephalosporinate P_1 (**14**). Methyl cephalosporinate P_1 (**1b**; 180 mg, m.p. 232–233·5°) was dissolved in a mixture of 1·5 ml Ac_2O and 2·5 ml pyridine. The soln was allowed to stand for 40 hr at room temp; addition of water then gave an amorphous ppt of **14** (60% yield). This ppt was collected, washed with water, and dried at 100° (0·01 mm) to remove traces of pyridine. The amorphous triacetate **14** failed to crystallize but showed a melting range of 101–103·5°. TLC of **14** on silica gel showed one spot having R_f 0·75 compared with R_f 0·62 for **1b**. The material from the spot, **14**, was purified with the aid of preparative TLC: IR ($CHCl_3$) 3500–3200 (intermolecular H-bonding OH), 2880, 1725, 1430, 1365, 1235, and 1015 cm^{-1}; mass spectrum peaks *m/e* 630, 570, and 441 corresponding to parent ion, M-69 and M-69-120; NMR $CDCl_3$ δ 0·81 (d, 3, J = 6 Hz) δ 4·58 (d, 1, J = 10 Hz) and δ 4·90 (bm, 1) corresponding to the Me protons at C-4 and the secondary protons at C-3 and C-6. The NMR data are shown in Table 1. (Found: C, 68·50; H, 8·84. $C_{36}H_{54}O_9$ requires: C, 68·54; H, 8·63%).

Acetylation of **2b**. Ac_2O (1 ml) was added to a soln of 100 mg **2b** in 3 ml pyridine all at room temp. After 4 hr the silica-gel TLC of the mixture showed 4 pronounced spots with R_f values 0·75, 0·68, 0·62, and 0·48. These same 4 spots persisted after 3 days of reaction. The reaction mixture was then poured into 100 ml water and the resulting light-yellow ppt was collected, washed with water and dried at 100° at a reduced press to remove traces of pyridine. An attempt to separate 38 mg of this mixture with a silica gel column was not successful. The mixture was tested again on a thin-layer plate and the spots having R_f 0·62 and 0·75 were identified as **1b** and **14** respectively through the comparison with the R_f values of authentic samples. The other spots were not identified. However, preparative thin-layer separation of these two spots gave amorphous samples which showed IR spectra identical with those of **1b** and **14** respectively.

Preparation of keto triacetate **15** *from the hydroxy triacetate* **14**. A soln of 100 mg of triacetate **14** (amorphous solid purified by preparative thin-layer plates) in 5 ml of AcOH was oxidized with 5% CrO_3 in 0·8 ml 95% AcOH as described for **11**. The residue failed to crystallize but was purified by preparative thin-layer plates coated with silica gel to give an analytically pure sample of **15**, IR (KBr) 2910, 1740, 1710, 1455, 1380, and 1250 cm^{-1}; $[\alpha]_{300}$ −853°, $[\alpha]_{317}$ −1025°, $[\alpha]_{325}$ −836°, and $[\alpha]_{340}$ −456° (*c* 0·235, 1 cm, EtOH). The NMR data are shown in Table 1. (Found: C, 68·27; H, 8·55. $C_{36}H_{52}O_9$ requires: C, 68·76; 8·34%).

Preparation of diketone **16** *from* **1b**. A soln of 100 mg of **1b**, m.p. 147–148°, in 5 ml AcOH was oxidized with 5% CrO_3 in 1·6 ml 95% AcOH as described for oxidizing **11**. The diketone **16** was not obtained in crystalline form but was purified by silica gel thin-layer plates: IR (KBr) 2910, 1740, 1725, 1710, 1450, 1380, and 1250 cm^{-1}; $[\alpha]_{310}$ −333°, $[\alpha]_{320}$ −516°, $[\alpha]_{325}$ −442°, and $[\alpha]_{340}$ −216° (*c* 0·120, 1 cm, EtOH). The NMR data are in Table 1. (Found: C, 67·09; H, 8·10. $C_{34}H_{43}O_8 \cdot H_2O$ requires: C, 67·75; H, 8·36%).

Clemmensen reduction of **16** *to* **17**. To a stirred soln of **16** (30 mg) in 1 ml 90% AcOH, 70 mg Zn dust was added in small portions during 15 min. After stirring at reflux temp for 60 min, the ppt was filtered out and the filtrate poured into water. The product was extracted with $CHCl_2$ and the extract was washed with water, dried, and evaporated to dryness *in vacuo*. The residue did not crystallize. Chromatography on silica gel thin-layer plates showed it contained a small amount of **16**. The ORD curve of **17** showed $[\alpha]_{310}$ −186°, $[\alpha]_{320}$ −398°, $[\alpha]_{325}$ −336°, and $[\alpha]_{340}$ −142° (*c* 0·113, 1 cm, EtOH).

Hydrogenation of **1a** *to dihydrocephalosporin* P_1 (**18a**). The procedure described for prep of **10a** was used which gave an 80% yield of **18a**, m.p. 153–154·5°, after crystallization from MeOH.

Hydrogenation of **1b** *to* **18b**. Methyl cephalosporinate (**1b**) was hydrogenated in the same manner used to prepare **10b** to give in 90% yield, after two recrystallizations from MeOH, **18b**; m.p. 207–209°; UV max (EtOH) 222 mμ (ε 7100).

Hydrogenation of **1a** *and* **1b** *to* **19a** *and* **19b**. Samples (50 mg) of **1a** and **1b** were hydrogenated separately as described for hydrogenation of **20b**. The hydrogenation products **19a** and **19b** were shown by TLC to be contaminated with **20a** and **20b** respectively.

Hydrogenation of **2a** *to* **20a** *and* **2b** *to* **20b**. A soln of **2b** (50 mg, m.p. 197–198·5°) in 20 ml abs EtOH and 0·5 ml AcOH was stirred at room temp under a H atm in the presence of 10 mg Adam's catalyst. After 1 hr, the catalyst was filtered out. A ppt appeared on pouring the filtrate into 100 ml water. Crystallization of the ppt from MeOH yielded 43 mg of **20b**; m.p. 170–171·5°; IR (KBr) 3630, 3570, 3500–3200, 2900, 2840, 1725, 1460, 1375, and 1260 cm^{-1}; mass spectrum *m/e* 550. The NMR spectrum no longer showed the peak corresponding to the C-24 proton of **1b**. The UV spectrum showed no absorption in the region of 220 mμ and a test with tetranitromethane was negative. (Found: C, 70·05; H, 9·92. $C_{32}H_{54}O_7$ requires: C, 69·78; H, 9·88%).

The same procedure was used to hydrogenate **2a** in 80% yield to **20a**, m.p. 234·237°, after crystallization from MeOH.

Acknowledgements—One of us (T.S.C.) is grateful to Eli Lilly and Company, Indianapolis, Indiana, for financial aid. We thank the Research Foundation, Oklahoma State University, for their assistance and Dr. O. C. Dermer for having read the manuscript. We are also indebted to Drs. H. Boaz and P. V. DeMarco for NMR determination and Messrs. W. C. Brown, H. C. Hunter, and D. C. Cline for their microanalytical assistance.

REFERENCES

[1] [a] T. S. Chou, E. J. Eisenbraun and R. T. Rapala, *Tetrahedron Letters* 409 (1967);
[b] R. T. Rapala, E. J. Eisenbraun and T. S. Chou, *Abstract of Papers, 152nd National Meeting, American Chemical Society* p. 045. New York, Sept. (1966).
[c] T. S. Chou, Ph.D. Thesis, Oklahoma State University, May (1967).
[2] Graduate Research Assistant at Oklahoma State University from 1963–1967.
[3] [a] H. B. Burton and E. P. Abraham, *Biochem. J.* **50**, 168 (1951);
[b] B. M. Baird, T. G. Halsall, E. R. H. Jones and G. Lowe, *Proc. Chem. Soc.* 257 (1961).
[4] T. G. Halsall, E. R. H. Jones and G. Lowe, *Proc. Chem. Soc.* 16 (1963).
[5] A. Melera, *Experientia* **19**, 566 (1963).
[6] L. P. Kuhn, *J. Am. Chem. Soc.* **74**, 2492 (1951).
[7] [a] L. Smith, *J. Chromatog.* **3**, 381 (1961);
[b] H. J. E. Loewenthal, *Tetrahedron* **6**, 269 (1959).
[8] T. G. Halsall, E. R. H. Jones, G. Lowe and C. E. Newall, *Chem. Comm.* 685 (1966).
[9] P. Oxley, *Ibid.* 729 (1966).
[10] [a] W. O.Godtfredsen and S. Vangedal, *Tetrahedron* **18**, 1029 (1962);
[b] W. O. Godtfredsen, W. von Doehne, S. Vangedal, A. Marquet, D. Arigoni and A. Melera, *Ibid.* **21**, 3505 (1965).
[c] A. Cooper, *Ibid.* **22**, 1379 (1966).
[11] D. Arigoni, W. von Doehne, W. O. Godtfredsen, A. Melera and S. Vangedal, *Experientia* **20**, 344 (1964).
[12] Y. Mazur, *J. Am. Chem. Soc.* **82**, 5889 (1960).
[13] S. M. Kupchan, P. Slade, R. J. Young and G. W. A. Miline, *Tetrahedron* **18**, 499 (1962).
[14] B. Capon, *Quarterly Rev.* **18**, 58 (1964); P. De Mayo, ed., *Molecular Rearrangements* (edited by P. De Mayo) p. 7121. Interscience, N.Y. (1963–1964).
[15] J. R. Holum, *J. Org. Chem.* **26**, 4814 (1961).

[16] J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta* **38**, 1529 (1955).
[17] S. Okuda, S. Iwasaki, K. Tsuda, Y. Sano, T. Hata, S. Udagawa, Y. Nakayama and H. Yamaguchi, *Chem. Pharm. Bull. Tokyo* **12**, 121 (1964).
[18] S. J. Angyal and C. G. MacDonald, *J. Chem. Soc.* 686 (1952).
[19] L. F. Fieser and M. Fieser, *Steroids*, Chap. 6; p. 216. Reinhold, New York (1959).
[20] C. Djerassi, ed., *Steroid Reactions* (Edited by R. C. Djerassi) p. 67. Holden-Day, San Francisco, California (1963).
[21] W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne and C. Djerassi, *J. Am. Chem. Soc.* **83**, 4013 (1961).
[22] N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry*, Chap. 8. Holden-Day, San Francisco, California, (1964).
[23] G. Slomp and F. MacKeller, *J. Am. Chem. Soc.* **82**, 999 (1960).
[24] T. J. Flautt and W. F. Erman, *Ibid.* **85**, 3212 (1963).
[25] P. J. van Dijck and P. de Somer, *J. Gen. Microbiol.* **18**, 377 (1958).
[26] R. Bucourt, M. Legrand, M. Vignau, J. Tessier and V. Delarogg, *C.R. Acad. Sci., Paris.* **257**, 2679 (1963).