The semicarbazone melted at 156.5–158.5° and a mixture melting point with the semicarbazone of the vinyl ketone was 154–162°.

Anal. Calcd. for $C_{16}H_{31}N_3O$: C, 68.28; H, 11.10. Found: C, 68.56; H, 10.91.

URBANA, ILL.

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Proof of the Structure and Stereochemistry of α-Amyrin by Synthesis from a β-Amyrin Derivative, Glycyrrhetic Acid[1,2]

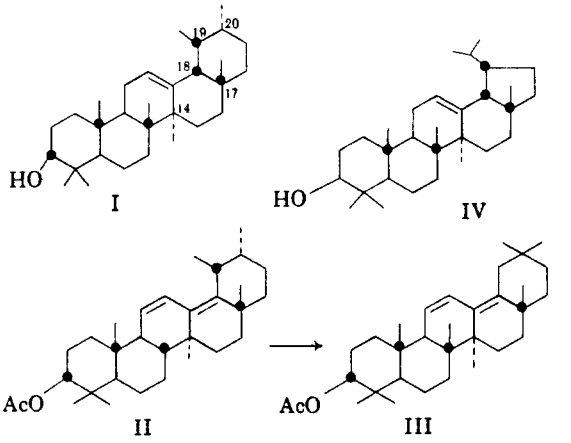
BY E. J. COREY AND E. W. CANTRALL

RECEIVED SEPTEMBER 27, 1958

A partial synthesis of α-amyrin according to the following sequence is described. Methyl glycyrrhetate → desoxoglycyrrhetic acid (VIb) → acetyl desoxoglycyrrhetic acid chloride (VIc) → 29-nor-20β-methylaminoölean-12-en-3β-ol (VIf) → 29-nor-20β-trimethylammonoiumolean-12-en-3-β-ol iodide (VIg) → 29-norolean-12:20(30)-dien-3β-yl acetate (VIIb) → 29,30-bisnorolean-12-en-20-on-3β-yl benzoate (VIIIb) → 30-norurs-12-en-20-on-3β-yl benzoate (IX) → ursa-12:20(30)-dien-3β-yl benzoate (X) → α-amyrin.

The gross structure of α-amyrin which had been determined by Ruzicka, Jeger and co-workers[3] (I without stereochemical connotations) was elaborated to the complete sterochemical description I, in 1954, on the basis of extensive chemical and physical data.[4] This stereoformula provided for the first time a reasonable explanation of several aspects of the chemistry of α-amyrin which had been puzzling (*e.g.*, the unreactivity of the $\Delta^{12,13}$ double bond in comparison with that in β-amyrin). In addition the considerations leading to the assignment of I focused attention on the fact that the D and E rings comprise a *cis*-decalin system which is more stable than the corresponding *trans*-decalin system formed by epimerization of C_{18}, an inversion in the usual order of stability. It is also noteworthy that formula I for α-amyrin agrees nicely with the Ruzicka–Eschenmoser scheme for triterpene biosynthesis and permits additional conclusions regarding the stereochemistry of hydrogen migration.[4b]

Subsequent to the proposal of stereoformula I two alternative formulations were advanced. The first of these, in which configurations opposite to those in I had been assigned to C_{17}, C_{19} and C_{20},[5] soon had to be discarded since it was discovered that acid-catalyzed isomerization of ursa-11:13-(18)-dien-3β-yl acetate (II) produces olean-11:13-(18)-dien-3β-yl acetate (known to be III),[6] a change which almost certainly does not affect the configuration at C_{17}. The other formulation[6,7] for α-amyrin (IV), which adopted the ring system of lupeol, seemed unlikely from the outset for reasons outlined previously (footnote 4, reference 2), and has now been exlcuded rigorously by the synthesis reported herein, which was commenced



in 1954, and by evidence presented in the interim by the Zurich group.[8]

Our work started with the view that since β-amyrin and I differ only with regard to substitution at C_{19} and C_{20}, a partial synthesis of α-amyrin could probably be achieved from a β-amyrin derivative possessing functionality at or near these centers by suitable degradation and reconstruction. The most advantageous starting material from this standpoint and because of its availability to us in the form of the glycoside was the substance glycyrrhetic acid (V), the structure[9] and stereochemistry[10] of which had been established.

It was soon apparent that the first task, the conversion of the glycoside, glycyrrhizinic acid,[11,12]

(1) Preliminary communication, THIS JOURNAL, **80**, 499 (1958).

(2) Taken fron the Ph.D. dissertation of E. W. Cantrall, University of Illinois, 1957.

(3) A. Meisels, O. Jeger and L. Ruzicka, *Helv. Chem. Acta*, **32**, 1075 (1949); see also O. Jeger, *Forts. Chem. Org. Naturs.*, **7**, 1 (1950).

(4) (a) E. J. Corey and J. J. Ursprung, *Chemistry & Industry*, 1387 (1954); (b) THIS JOURNAL, **78**, 183 (1956); (c) previously the configurations at C_9, C_{14}, C_{17}, C_{18}, C_{19} and C_{20} were unknown, although the configuration at C_{17} opposite to that in I had been considered as proved on the basis of lengthy degradative sequences [O. Jeger, *Angew. Chem.*, 196 (1951); see also *Ann. Rep.*, **48**, 198 (1951)].

(5) J. L. Beton and T. G. Halsall, *Chemistry & Industry*, 1560 (1954).

(6) G. G. Allen, J. M. Beaton, J. I. Shaw, F. S. Spring, R. Stevenson, J. L. Stewart and W. S. Strachan, *ibid.*, 281 (1955).

(7) F. A. Spring and co-workers, *J. Chem. Soc.*, 2606, 2610, 3072, 3371, 3378, 3992 (1955); 456, 465 (1956); see also D. D. Phillips and D. E. Tuites, THIS JOURNAL, **78**, 5438 (1956) and G. D. Meakins, *Chemistry & Industry*, 1353 (1955).

(8) A. Malera, D. Arigoni, A. Eschenmoser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **39**, 441 (1956), succeeded in dissecting ring E from α-amyrin and in converting the fragment so obtained into a 2,3,6-trimethylcyclohexanone of known absolute configuration thereby establishing unambiguously the configuration at C_{18} in α-amyrin and the fact that the E ring is six membered.

(9) L. Ruzicka and A. Marxer, *ibid.*, **22**, 195 (1939).

(10) (a) D. H. R. Barton and N. J. Holness, *J. Chem. Soc.*, 78 (1952); (b) J. M. Beaton and F. S. Spring, *ibid.*, 3126 (1955).

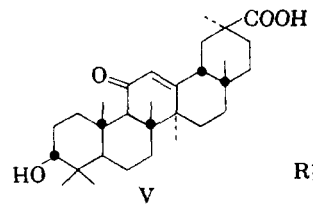
(11) Glycyrrhizinic acid, $C_{42}H_{62}O_{16}$, is a β-glycoside containing two hexuronic acid units: (a) A. Tschirch and H. Cederberg, *Arch. Pharm.*, **245**, 97 (1907); (b) W. Voss, P. Klein and H. Sauer, *Ber.*, **70**, 122 (1937); (c) C. Norman, *Chem. Weekblad*, **48**, 213 (1952).

(12) We are indebted to Dr. C. K. Swift of the MacAndrews–Forbes Co. for generous supplies of glycyrrhizinic acid as the monoammonium salt.

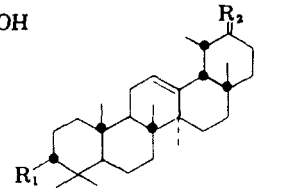
to pure glycyrrhetic acid requires considerable care, a fact which was also indicated by the widely divergent physical constants reported for glycyrrhetic acid[11a,11b,13-20] from acid-catalyzed hydrolysis of the glycoside under a variety of conditions. In our own experiments the difficulties in obtaining pure glycyrrhetic acid from the hydrolysis seem to stem from the occurrence of isomerization at C_{18} to the more stable 18-iso (D/E *trans*) configuration, a known transformation of Δ^{12}-11-ketones in the β-amyrin series[10a] which has recently been observed with glycyrrhetic acid itself.[10b] This would also account for the variation in properties reported previously. Hydrolysis of the glycoside with 1% aqueous sulfuric acid in an autoclave at 130°,[11a,11b,13-15,19] seems to minimize epimerization at C_{18} and to afford a much purer product than that obtained with either concentrated hydrochloric acid[15,16] or methanolic hydrogen chloride,[16,17,20] although further purification was necessary. The procedure developed for obtaining pure glycyrrhetic acid involved esterification of the crude acid with diazomethane followed by partial saponification with 3% methanolic potassium hydroxide for 2.5 hours to remove the more readily hydrolyzed methyl 18-iso-glycyrrhetate.

Hydrogenation of methyl glycyrrhetate with high-activity platinum oxide (Baker and Co., activity 535)[4a] in acetic acid produced methyl 11-desoxoglycyrrhetate (VIa)[21] which after vigorous saponification (120 hours reflux with 1:4 toluene–15% ethanolic potassium hydroxide)[22] yielded 11-desoxoglycyrrhetic acid (VIb). Acetylation of VIb followed by reaction with phosphorus pentachloride in dixoane produced acetyl desoxyglycyrrhetic acid chloride (VIc) which was converted *via* the azide VId to the isocyanate VIe.[23] The isocyanate VIe was reduced by lithium aluminum hydride in tetrahydrofuran at reflux to 29-nor-20β-methylaminoölean-12-en-3β-ol (VIf) with an over-all yield for the process VIb → VIf of 83%.

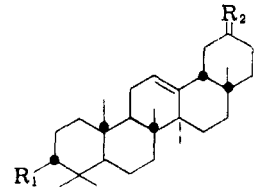
The next stage of the synthesis, the removal of the remaining carbon at C_{20}, was carried out from the amine VIf in four steps. Reaction of the amine with methyl iodide afforded the crystalline methiodide VIg which was transformed into the exocyclic olefin VIIa by treatment with potas-

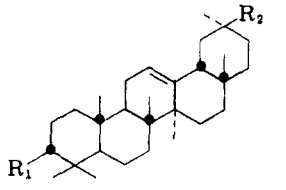


V



IX, R_1 = OBz, R_2 = O
X, R_1 = OBz, R_2 = CH_2





VIIa, R_1 = OH, R_2 = CH_2
VIIb, R_1 = OAc, R_2 = CH_2
VIIIa, R_1 = OAc, R_2 = O
VIIIb, R_1 = OBz, R_2 = O

VIa, R_1 = OH, R_2 = $COOCH_3$
VIb, R_1 = OH, R_2 = COOH
VIc, R_1 = OAc, R_2 = COCl
VId, R_1 = OAc, R_2 = CON_3
VIe, R_1 = OAc, R_2 = N=C=O
VIf, R_1 = OH, R_2 = $NHCH_3$
VIg, R_1 = OH, R_2 = $(CH_3)_3N^+$

sium *t*-butoxide in *t*-butyl alcohol. Structure VIIa was indicated by the appearance of strong terminal methylene absorption at 887–890 cm.$^{-1}$ in the infrared spectrum of the elimination product and also that of the corresponding acetate (VIIb). The choice of potassium *t*-butoxide was dictated by the axial orientation of the 20-trimethylammonium group in VIg which allows the possibility of elimination to give endocyclic olefin as well as the exocyclic olefin VIIa. It was anticipated that with a large base such as *t*-butoxide removal of the 19α- or 21α-hydrogen would be far less favorable for steric reasons than removal of a proton from the relatively accessible methyl group at C_{20}. In addition the combination of *t*-butoxide–*t*-butyl alcohol reduces the possibility of an E_1-type elimination. As might be expected from the above considerations, it was found that sodium methoxide in methanol was not a satisfactory reagent for the production of the exocyclic olefin VIIa from the quaternary salt VIf and that a mixture of olefins results under these conditions.

Selective hydroxylation of the E-terminal double bond in VIIb was easily accomplished by reaction with osmium tetroxide since the 12,13-double bond is quite unreactive to this reagent under mild conditions. The resulting diol underwent glycol fission smoothly upon exposure to neutral periodate and 29,30-bisnorolean-12-en-20-one-3β-yl acetate (VIIIa) was formed in 65% yield over-all from VIIb. The structure of this substance is clearly supported by the analytical data and the infrared spectrum which exhibits strong absorption maxima at 1724 (C_{20}-carbonyl) and 1740 cm.$^{-1}$ (C_3-acetate).

It is of importance that the bisnor ketone VIIIa still possesses the *cis* D/E fusion characteristic of the β-amyrins, a result which follows from the fact that in no stage of its synthesis had the hydrogen at C_{18} been labilized to allow epimerization

(13) F. Bergmann, *Biochem. Z.*, **267**, 296 (1933).

(14) P. Karrer, W. Karrer and J. C. Chao, *Helv. Chim. Acta*, **4**, 100 (1921).

(15) L. Ruzicka and H. Leuenberger, *ibid.*, **19**, 1402 (1936).

(16) L. Ruzicka, M. Furter and H. Leuenberger, *ibid.*, **20**, 312 (1937).

(17) W. Voss and G. Butter, *Ber.*, **70**, 1212 (1937).

(18) T. Kariyone and O. Nonaka, *J. Pharm. Soc. Japan*, **57**, 166 (in English 20-4) (1937).

(19) K. Takeda, *ibid.*, **58**, 731 (1938).

(20) P. Bilham, G. A. R. Kon and W. C. J. Ross, *J. Chem. Soc.*, 535 (1942).

(21) L. Ruzicka, H. Leuenberger and H. Schellenberg, *Helv. Chim. Acta*, **20**, 1271 (1937).

(22) The fact that these conditions are necessary for complete hydrolysis whereas methyl glycyrrhetate is totally saponified by heating to reflux for 22 hours with 0.5 *N* alcoholic potassium hydroxide[18] suggests that in the latter case prior epimerization at C_{18} takes place to give the more readily hydrolyzed methyl 18-*iso*-glycyrrhetate which subsequently leads to 18-*iso*-glycyrrhetic acid. Unfortunately, no physical constants were reported[18] for the saponification product.

(23) For an analogous transformation in the abietic acid series see H. H. Zeiss and W. B. Martin, THIS JOURNAL, **75**, 5935 (1953).

at that center. Thus, the bisnor ketone must be a *cis*-decalone which is analogous structurally to coprostanone with respect to the environment of the carbonyl group. Consequently, enolization of the bisnor ketone would be expected to involve C_{19} rather than C_{21} for the same reasons which direct the enolization of coprostanone toward C_4 rather than C_2, *viz.*, the greater steric acceleration for enolization toward the α-carbon which is axial to the adjacent ring. It seemed likely from these considerations that the bisnor ketone could be methylated selectively at C_{19}.

This surmise proved to be correct and, indeed, the 19β-methylated bisnor ketone was easily obtained in good yield even on a small scale. The 3-benzoyl derivative (VIIIb) of VIIIa, in dry dioxane solution, was treated with an ethereal solution of triphenylmethyl sodium to form the sodio derivative (essentially instantaneously) until a small excess was present as indicated by the persistence of its characteristic red color. A large excess of methyl iodide was then added to effect methylation. It is noteworthy that the excess triphenylmethyl sodium is destroyed instantaneously by methyl iodide, presumably with formation of triphenylethane. Under these conditions the methylated ketone IX was produced efficiently and could be isolated pure in 67% yield.

The β-(equatorial) orientation of the newly attached methyl group was apparent from the resistance of the methylation product to isomerization. Actually, the 19α-methyl derivative of VIIIb is so greatly strained because of interaction with the 14α-methyl substituent that its formation, even transiently, seems unlikely. That the new methyl substituent was, in fact, at C_{19} rather than at C_{21}, the less likely alternative, was shown clearly by optical rotatory dispersion measurements.[24] The optical rotatory dispersion curves of the bisnor ketone VIIIb and its methylated derivative IX were both of the coprostan-3-one type.[25] Since 4β-methyl-coprostan-3-one exhibits a dispersion curve of shape identical with that of coprostan-3-one, and 2β-methylcoprostan-3-one shows an *inverted* curve, the product of methylation of the bisnor ketone VIIIb must correspond in the vicinity of the carbonyl group to 4β-methylcoprostan-3-one. It must therefore be the 19β-methylated ketone IX.

It should be noted here that the methylation procedure employed above is probably generally applicable to the monomethylation of ketones and seems especially applicable for small scale operations. The enolate is formed rapidly and completely under mild conditions and can then be subjected to methylation with a high concentration of active methylating reagent to minimize dialkylation. Apparently acetate groups interfere with the process as evidenced by the fact that the acetoxy bisnor ketone VIIIa gave an oily mixture, in contrast to the benzoate VIIIb.

The introduction of the last carbon atom was accomplished by treating IX with triphenylphosphine-methylene[26] under the conditions recently described for steroids.[27] The infrared absorption spectrum of the product displayed a sharp maximum at 888 cm.$^{-1}$ confirming the presence of a methylene group at C_{20} in the product as in X. The conversion of the 20-methylene substituent to a 20α-(equatorial) methyl group, the final alteration required to produce structure I, was then undertaken.

From the outset the method of choice for this transformation seemed to be chemical reduction using an alkali metal–proton donor combination since terminal methylene groups are readily reduced by such chemical methods[28] and since the substituents produced by reduction of an exocyclic (or endocyclic) double bond generally possess the equatorial orientation. For example, the reduction of cyclohexanones by sodium-proton donor reagents produces the equatorial alcohols. Similarly, we have observed that the reduction of 3-methylenecholestane with lithium–ethylenediamine produces 3β-methylcholestane very selectively and that the same reagent reduces 3-deuterio-Δ^2-cholestene and 3-deuterio-3α- or 3β-bromocholestane predominantly (>90%) to 3β-deuteriocholestane.[29] In addition, several analogous results have been reported recently.[30-32] In fact, the selective reduction of the olefinic linkage at C_{20} in X proceeded smoothly using lithium–ethylenediamine[33,34] to form the desired product I which was isolated as the acetate. This synthetic material was identical with authentic α-amyrin acetate as was apparent from melting points and mixture melting point and the complete correspondence of infrared spectra, X-ray powder diffraction patterns and optical rotatory disperison curves.

This work constitutes a partial synthesis of α-amyrin from a β-amyrin derivative of known constitution and stereochemistry and, further, is of such a nature as to demonstrate unequivocally the correctness of expression I for α-amyrin.

It is a pleasure to thank the Ethyl Corp. and the Proctor and Gamble Co. for fellowships and the Alfred P. Sloan Foundation and the National Science Foundation for additional financial support.

Experimental[35]

Crude Glycyrrhetic Acid from Monoammonium Glycyrrhizinate (V).—Ten 1-liter flasks, each containing 4.95 g. of

(24) Obtained through the courtesy of Drs. C. Djerassi and E. J. Eisenbraun.

(25) C. Djerassi and W. Closson, THIS JOURNAL, **78**, 3761 (1956).

(26) G. Wittig and U. Schöllkopf, *Ber.*, **87**, 1318 (1954).

(27) F. Sondheimer and R. Mechoulam, THIS JOURNAL, **79**, 5029 (1957).

(28) In contrast, it has been observed by Dr. G. Gregoriou in these laboratories that the 12,13-double bond of β-amyrin is not reduced even under drastic conditions, *e.g.*, excess lithium–ethylenediamine at reflux. The inertness of the 12,13-double bond to chemical reduction was, of course, desirable for the case at hand.

(29) G. A. Gregoriou, Ph.D. Thesis, University of Illinois, 1958.

(30) D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.*, 3045 (1954).

(31) G. Roberts and C. W. Shoppee, *ibid.*, 3418 (1954).

(32) D. H. R. Barton, A. Campos-Neves and R. C. Cookson, *ibid.*, 3500 (1956).

(33) L. Reggel, R. A. Friedel and I. Wender, *J. Org. Chem.*, **22**, 891 (1957).

(34) See also R. A. Benkeser, G. Schroll and D. M. Sauve, THIS JOURNAL, **77**, 3378 (1955), for reduction of olefins using lithium–ethylamine.

(35) All melting points are corrected and, unless stated otherwise, were taken in open soft-glass capillaries. Infrared spectra were obtained with a Perkin–Elmer model No. 21 infrared spectrophotometer.

pure monoammonium glycyrrhizinate (provided by the MacAndrews-Forbes Co.) and 500 ml. of 1% aqueous sulfuric acid were placed in a sterilizer and heated for 22 hours at 24 p.s.i. 129°. The crude brown aglycone was filtered, washed with *ca.* 2 l. of water and dried overnight at 80°, to yield 25.91 g. (93.5%) of crude glycyrrhetic acid. The acid was not purified as such, but was converted to its methyl ester.

Methyl Glycyrrhetate.—The crude hydrolysate obtained from the hydrolysis of monoammonium glycyrrhizinate (25.91 g.) was treated with chloroform and filtered to remove any glycoside still present. A black residue estimated to be less than 1 g. was obtained. On evaporating the chloroform solution to dryness, a red glass weighing 25.66 g. (92.8%) was obtained. A solution of the crude acid in *ca.* 500 ml. of methanol was treated with an excess of ethereal diazomethane and let stand 3 hours at room temperature. The mixture was concentrated on a steam-bath until crystals began to appear and was set aside to cool. The product was filtered, rinsed with a little methanol and dried at 80° to give 15.42 g. (58.3%) of colorless needles, m.p. *ca.* 233–249°, $[\alpha]^{26.5}D$ +151.7° (*c* 2.49, α +3.78°). Additional methyl glycyrrhetate was obtained by pouring the above liquors into an equal volume of 5% potassium hydroxide, diluting the resulting slurry to twice its volume with water, and extracting the product into ether. The extract was worked up in the usual way, yielding 7.13 g. (27%) of yellow needles, $[\alpha]^{27.5}D$ +119.3° (*c* 7.36, α +8.78°).

The crude methyl glycyrrhetate obtained from a number of preparations contained varying amounts of its epimer, methyl 18-*iso*-glycyrrhetate. The melting points varied within the range *ca.* 215–245°, and the specific rotations varied from *ca.* +120 to 150°. The first crops obtained always had high specific rotations, varying from *ca.* +140 to 153°. In order to maximize the yields of pure methyl glycyrrhetate, the crude ester was partially saponified by heating *ca.* 3.0-g. portions of the crude ester to reflux for 2.5 hours with 3% aqueous methanolic potassium hydroxide (29 g. of potassium hydroxide pellets, 80.8 g. of water and 728 g. of methanol) under nitrogen. The reaction mixtures were diluted with an equal volume of water (*ca.* 750 ml.), and extracted repeatedly with ether. The extracts were worked up in the usual way. The products were washed with *n*-heptane and dried at 80°. Methyl glycyrrhetate obtained from a number of partial saponification experiments ($[\alpha]D$ +152 to +156°) was recrystallized once from methylene chloride–methanol, m.p. 242.5–249.5°, $[\alpha]^{29}D$ +153.2° (*c* 1.41, α +2.16°); infrared absorption in 10% chloroform: 1725 (s) cm.$^{-1}$ for the ester carbonyl; 1655(s) cm.$^{-1}$ for the conjugated carbonyl; ultraviolet absorption: λ_{max} 249 mμ, log ϵ 4.15 (*c* 0.056 mg./ml. in 95% ethanol).

Methyl Glycyrrhetate Acetate.—A solution of 1.57 g. of methyl glycyrrhetate (m.p. 242.5–249°, $[\alpha]D$ +153.2°) in 11 ml. of pyridine (dried over calcium hydride) was heated on a steam-bath for 45 min. with 15 ml. of acetic anhydride and then stored at room temperature for 20 hours. The excess acetic anhydride was decomposed with methanol, and the mixture was concentrated to dryness *in vacuo* on a steam-bath. The product was extracted into ether and worked up in the usual way. Two recrystallizations from methylene chloride–methanol yielded 1.40 g. (82%) of colorless plates, m.p. 304.5–305.5°, $[\alpha]^{24}D$ +140.1° (*c* 1.57, α +2.20°); infrared absorption in 10% chloroform: 1722(s) cm.$^{-1}$ for ester carbonyl, 1655(s) cm.$^{-1}$ for conjugated carbonyl, 1258(s) cm.$^{-1}$ for acetate; ultraviolet absorption: λ_{max} 248 mμ, log ϵ 4.10 (*c* 0.076 mg./ml. in 95% ethanol).

Methyl Desoxoglycyrrhetate (VIa).—To a solution of 10.01 g. of methyl glycyrrhetate (m.p. 245–251°, $[\alpha]D$ +152.1°) in 800 ml. of glacial acetic acid was added 1.00 g. of platinum oxide (Baker and Co. activity no. 535; platinum oxide prepared at 520° according to Frampton[36] was also suitable for the reduction). The mixture was shaken for 7 hours at room temperature and atmospheric pressure under an atmosphere of hydrogen. The catalyst was filtered off, and the colorless filtrate was concentrated to dryness *in vacuo* on a steam-bath. The product[21] was crystallized once from chloroform–methanol to give 9.24 g. (93.7%) of colorless needles, m.p. 233–245°, $[\alpha]^{30}D$ +115.4° (*c* 1.23, α +1.42°); infrared absorption in 5% chloroform: 1725(s) cm.$^{-1}$ for ester carbonyl; no conjugated carbonyl band was present.

Methyl Desoxoglycyrrhetate Acetate.—A solution of 200 mg. of methyl desoxoglycyrrhetate (m.p. 237.5–241°) in 10 ml. of pyridine (dried over calcium hydride) was heated on a steam-bath with 10 ml. of acetic anhydride for 2 hours and let stand for 24 hours at room temperature. The product[20,21] was worked up in the usual way and crystallized from chloroform–methanol to give 192 mg. (88%) of fine colorless crystals, m.p. 258–260.5°. Another preparation had m.p. 262–263°, $[\alpha]^{29}D$ +112.3° (*c* 1.06, α +1.19°); infrared absorption in 10% chloroform: 1722(s) cm.$^{-1}$ for ester carbonyl, 1258(s) cm.$^{-1}$ for acetate.

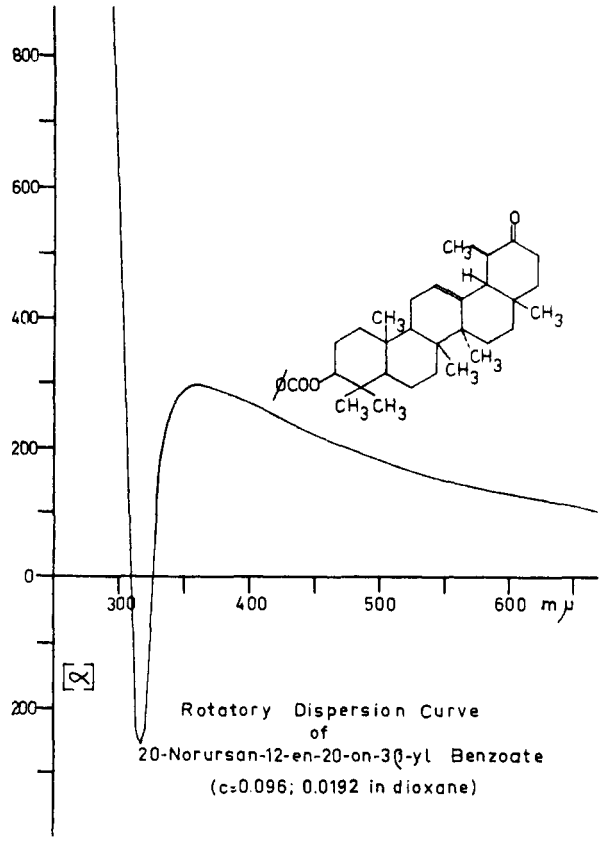
Desoxyglycyrrhetic Acid Acetate.—A solution of 9.00 g. of methyl desoxoglycyrrhetate (m.p. 233–245°, $[\alpha]^{30}D$ +115.4°) in 300 ml. of toluene was treated with 450 ml. of a 15% solution of potassium hydroxide in ethanol and heated to reflux in a copper flask for 120 hours under nitrogen. The mixture was poured into a beaker containing 500 g. of ice and 350 ml. of concentrated hydrochloric acid; the product was extracted into ether, washed several times with dilute sodium bicarbonate solution, and then worked up in the usual way to give a deposit of glistening white needles. The crude acid was dissolved in 100 ml. of pyridine and was treated with 100 ml. of acetic anhydride and heated for 1 hour on a steam-bath. After standing 24 hours at room temperature, the reaction mixture was worked up in the usual way, and the product[9] was crystallized from chloroform–methanol to provide 8.41 g. (88.3%) of glistening colorless plates, m.p. 305–307° with slight decomposition, $[\alpha]^{29}D$ +117.2° (*c* 0.93, α +1.09°); infrared absorption in 10% chloroform: 1724(s) cm.$^{-1}$ for acetate carbonyl, 1705(s) cm.$^{-1}$ for acid carbonyl, 1262(s) cm.$^{-1}$ for acetate, 2400–2800 cm.$^{-1}$ for carboxyl, 3600(w) cm.$^{-1}$ for hydroxyl.

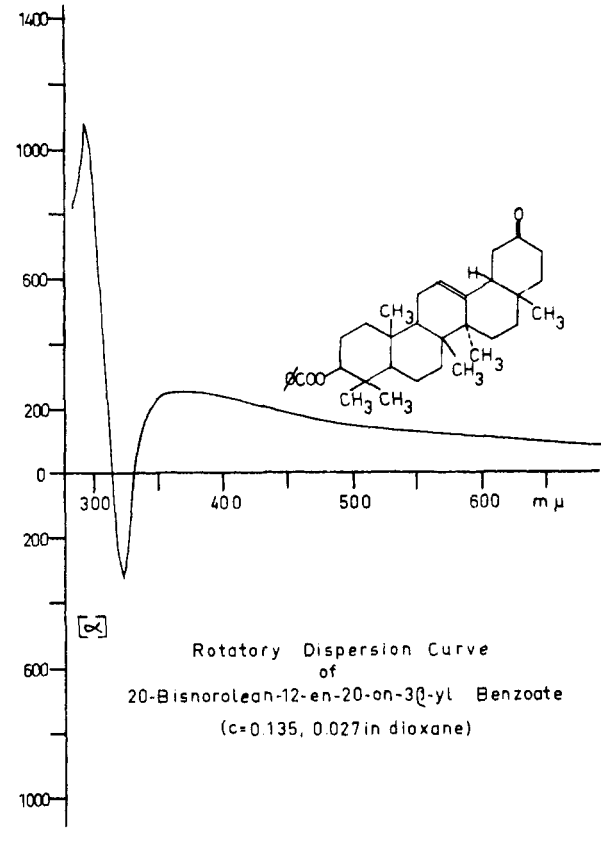
Acetyl Desoxoglycyrrhetic Acid Chloride.—A solution of 104 mg. of acetyl desoxoglycyrrhetic acid (m.p. 302.8–304.8°) in 5 ml. of absolute dioxane was treated with 133 mg. of phosphorus pentachloride and heated for 2 hours on a steam-bath. The solvent was stripped off *in vacuo* at room temperature, and the residue was taken up in ether, washed with 1% potassium hydroxide solution, and then worked up in the usual way. The product (VIc)[9] crystallized from *n*-hexane in clusters of colorless needles to yield 50.5 mg. (46.5%) of product; m.p. slight softening and sintering at 243°, decomposes and softens up to 297°, completely fluid at 304°. Evaporation of the liquors from the first crop afforded an additional 53.4 mg. of yellowish amorphous solid whose infrared absorption spectrum was nearly identical with that of the first crop. Both fractions showed a strong acid chloride band at 1787 cm.$^{-1}$ (Nujol) which was fairly broad. From one or two experiments a product melting from 248–251° was obtained; however, it was subsequently found unnecessary to purify extensively the crude product.

29-Nor-20β-methylaminoölean-12-en-3β-ol (VIf).—To a solution of 8.01 g. of desoxoglycyrrhetic acid acetate in 200 ml. of absolute dioxane was added 6.67 g. of phosphorus pentachloride. The mixture was heated for 2 hours on a steam-bath and then left to stand at room temperature for 17 hours. The dioxane was removed *in vacuo* at 50–60°, leaving a deposit of colorless needles. The residue was triturated several times with anhydrous acetone, and the solvent was removed *in vacuo* at 50–60°. The crude acid chloride was dissolved in 1750 ml. of anhydrous acetone and was transferred to a 3-liter 3-neck flask fitted with a mechanical stirrer. A solution of 10.41 g. of sodium azide in 40 ml. of water was added with stirring, and the slurry was stirred for 3 hours at room temperature. The reaction mixture was diluted with 2500 ml. of water, and the acid azide VId was extracted into xylene. The xylene extract was washed several times with saturated sodium chloride solution, dried for 2 hours over anhydrous magnesium sulfate, and heated to reflux for 1.5 hours. On concentrating the xylene solution to dryness *in vacuo* on a steam-bath, a yellow-orange semicrystalline solid was obtained. An infrared spectrum of the crude isocyanate (VIe) in 10% chloroform showed the following bands: 2265(s) cm.$^{-1}$ for the isocyanate group, 1725(s) and 1258(s) cm.$^{-1}$ for the acetate function. The crude isocyanate was dissolved in 250 ml. of tetrahydrofuran (distilled from sodium and stored over sodium wire and cal-

All specific rotations were measured in chloroform. The term "in the usual way" refers to extracts and indicates that they were washed successively with water and saturated sodium chloride solution, filtered through cotton, and concentrated to dryness. Microanalyses were by Mr. J. Nemeth and associates.

(36) V. L. Frampton, J. D. Edwards and H. R. Henze, THIS JOURNAL, **73**, 4432 (1951).





cium hydride) and heated to reflux for 90 hours with 8.00 g. of lithium aluminum hydride. The reaction mixture was cooled to room temperature, and the excess hydride was decomposed by the cautious addition of water. The product was extracted into ether–methylene chloride (*ca.* 2:1) and was worked up in the usual way. One recrystallization from pyridine–water afforded 5.88 g. (83% based on desoxoglycyrrhetic acid acetate) of yellow-white needles, m.p. bulk 212–226.5°, completely fluid at 229.5°, with slight decomposition. A sample for analysis was crystallized several more times from pyridine–water, and dried for 48 hours at 0.7 mm. (98°); m.p. 216–229.5°, $[\alpha]^{31}D$ +99° (*c* 1.06, α +1.05).

Anal. Calcd. for $C_{30}H_{51}ON$: C, 81.57; H, 11.64; N, 3.17. Found: C, 81.07; 81.29; H, 11.88, 11.90; N, 3.32, 3.40.

The rather wide m.p. range of VIf may be due to contamination by a small amount of the corresponding primary amine which might also have been formed in the conversion VId → VIf.

29-Nor-20β-trimethylammoniumolean-12-en-3-β-ol Iodide (VIg).—A solution of 4.00 g. of 20-nor-20β-methylaminoolean-12-en-3-β-ol in 1000 ml. of absolute ethanol was heated to reflux with 80.00 g. of anhydrous potassium carbonate and 129 g. of methyl iodide for 15 hours. The mixture was concentrated to near dryness *in vacuo*, slurried into a separatory funnel with water, extracted into methylene chloride, and worked up in the usual way. The product was crystallized from methylene chloride–ether to provide 4.88 g. (90.2%) of fine colorless plates. The product gave a positive Beilstein test for halogen.

29-Nor-olean-12:20(30)-dien-3β-ol (VIIa).—To a solution of 250 ml. of freshly prepared potassium *t*-butoxide in *t*-butyl alcohol (1.1 *M*) was added 1.77 g. of 20-nor-20β-trimethylammoniumolean-12-en-3β-ol iodide, and the mixture was heated to reflux for 21.5 hours under nitrogen. The mixture was concentrated to dryness *in vacuo* on a steam-bath, and the product was extracted into ether and worked up in the usual way. One recrystallization from methylene chloride–methanol afforded 905 mg. (67.6%) of colorless needles, m.p. 166.5–168.1°. An infrared spectrum of the product in 10% chloroform showed a strong band at 890 cm.$^{-1}$, characteristic of a terminal methylene group in addition to a band of moderate intensity at 1652 cm.$^{-1}$. After several recrystallizations from methylene chloride–methanol and one from acetone–water, the product melted from 166–169°. A sample for analysis was dried for 18 hours at 0.2 mm. (69°); $[\alpha]^{27}D$ +156.6° (*c* 0.53, α +0.83°).

Anal. Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29. Found: C, 84.28; H, 11.21.

An additional 365 mg. (28.4%) of product, m.p. 141–165°, was obtained by concentrating the liquors from the first crop to dryness. Upon acetylation with acetic anhydride and pyridine, followed by two recrystallizations from methylene chloride–methanol, 207 mg. (51.5%) of find colorless crystals of the olefin acetate was obtained, m.p. 178–185.5°.

29-Nor-olean-12:20(30)-dien-3-β-yl-Acetate (VIIb).—A solution of 300 mg. of 20-nor-olean-12:20(29)-dien-3β-ol (m.p. 166.5–168.1°) in 2 ml. of pyridine was treated with 2 ml. of acetic anhydride, heated for a few minutes on a steam-bath, and let stand at room temperature for 15 hours. The product was worked up in the usual way and crystallized from methylene chloride–methanol to give 256 mg. (77.5%) of colorless plates, m.p. 193.5–195.3°; infrared absorption in 10% carbon disulfide: 887(s), 1652(m) cm.$^{-1}$ for terminal methylene; 1737(s), 1247(s) cm.$^{-1}$ for acetate. After several recrystallizations from acetone–water, the melting point was raised to 196.5–197.5°, and the product was dried for 18 hours at 0.2 mm. (69°) and submitted for analysis; $[\alpha]^{26}D$ +163.6°, +164.1° (*c* 0.55, α +0.90°; *c* 0.78, α +1.28°).

Anal. Calcd. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found: C, 82.42; H, 10.80.

29,30-Bisnorolean-12-en-20-on-3β-yl Acetate (VIIIa).—To a solution of 2.50 g. of 20-norolean-12:20(29)dien-3β-yl acetate in 125 ml. of absolute ether was added 100 drops of pyridine and 1.538 g. of osmic acid. The flask was stoppered and let stand at room temperature for 17.5 hours, during which time the osmate ester precipitated as a mass of dark needles. The solvents were removed *in vacuo* at room temperature, and the osmate ester was taken up in 500 ml. of 95% ethanol and transferred to a 3-liter 3-neck flask fitted with a mechanical stirrer. A solution of 6.98 g. of sodium sulfite in 115 ml. of water was added, and the mixture was heated to reflux for 3 hours with stirring. The mixture was

stirred an additional 25 minutes at room temperature, the osmium was filtered through Filter-cel, and the colorless filtrate was concentrated to dryness *in vacuo* on a steam-bath. The residue was extracted into ether and worked up in the usual way. The product, an amorphous white solid, was dissolved in 625 ml. of 95% ethanol and treated with a solution of 2.61 g. of sodium periodate (meta)[37] in 25 ml. of water. The colorless solution was allowed to stand 50 hours at room temperature in the dark, following which it was diluted with 700 ml. of water and extracted repeatedly with ether–methylene chloride (2:1). The extract was worked up in the usual way, and the crude product was further dried overnight in a 110° oven. The crude product was acetylated with a mixture of 25 ml. of pyridine and 25 ml. of acetic anhydride and worked up in the usual way. Two recrystallizations from acetone–water afforded 1.62 g. (64.6%) of VIIIa as long colorless needles, m.p. 228.5–240°, with slight decomposition. A sample for analysis was recrystallized several times from acetone–water and dried for 24 hours at 0.5 mm. (98°); m.p. 240.5° drop formed, 242.5–244° bulk melted with slight decomposition (Pyrex capillary), $[\alpha]^{29}D$ +85.5° (*c* 0.83, α +0.71°); infrared absorption in 5% carbon disulfide: 1724(s) cm^{-1} for ketone carbonyl; 1740(s), 1250(s) $cm.^{-1}$ for acetate.

Anal. Calcd. for $C_{30}H_{46}O_3$: C, 79.24; H, 10.20. Found: C, 79.22; H, 10.22.

29,30-Bisnorolean-12-en-20-on-3β-yl Benzoate (VIIIb).—A solution containing 732 mg. of 20-bisnorolean-12-en-20-on-3β-yl acetate (m.p. 228.5–240°), 50 ml. of absolute dioxane, 125 ml. of reagent grade methanol and 12.42 g. of potassium hydroxide pellets[38] was heated to reflux for 3.75 hours under nitrogen. The mixture was concentrated to dryness *in vacuo* on a steam-bath, and the product was extracted into ether and worked up in the usual way. The product was dried for 1 hour at 80°, dissolved in *ca.* 15 ml. of pyridine, and treated with 339 mg. of benzoyl chloride. The resulting solution was stoppered and let stand for 4 days at room temperature. The mixture was diluted with a few ml. of water and stirred for 24 hours at room temperature, concentrated to dryness *in vacuo* on a steam-bath, extracted into ether–methylene chloride (2:1), washed with dilute hydrochloric acid and dilute sodium hydroxide, and worked up in the usual way. The slightly colored semi-crystalline product was dissolved in benzene, placed on a 10-g. column of alumina, and eluted with 400 ml. of benzene. The product crystallized from *ca.* 20 ml. of hot glacial acetic acid by the dropwise addition of water to yield 366 mg. (44%) of colorless needles, m.p. 231–233.5° (Pyrex capillary); infrared absorption in 5% carbon tetrachloride: 1720(s) $cm.^{-1}$ for both the benzoate and ketone carbonyls, 1277(s) $cm.^{-1}$ for benzoate, 712(s) $cm.^{-1}$ for monosubstituted phenyl. A sample for analysis was crystallized once more from acetic acid–water and dried for 48 hours at 0.7 mm. (98°); m.p. 229.5–231.5°, $[\alpha]^{31}D$ +100° (*c* 0.58, α +0.58°).

Anal. Calcd. for $C_{35}H_{48}O_3$: C, 81.35; H, 9.36. Found: C, 81.48; H, 9.70.

30-Norurs-12-en-20-on-3β-yl Benzoate (IX).—Into a stirred solution of 101 mg. of 20-bisnorolean-12-en-20-on-3β-yl benzoate (m.p. 228.5–231.5°) in 20 ml. of absolute dioxane under an atmosphere of nitrogen was injected 4.0 ml. of ethereal triphenylmethylsodium[39] solution (*ca.* 0.13–0.15 *N*). A distinct red color persisted, indicating that a slight excess of the base was present. The mixture was stirred for a few minutes at room temperature, and 2.0 ml. of methyl iodide was injected into the reaction mixture. After stirring 15 hours at room temperature, the reaction mixture was neutral to litmus, and it was concentrated to dryness *in vacuo* on a steam-bath, extracted with ether, and worked up in the usual way. The crude product, a mixture of triphenylmethane and the alkylated ketone, was dissolved in a few ml. of benzene and placed on a 15-g. column of alumina. Triphenylmethane (*ca.* 150 mg.) was eluted with 180 ml. of *n*-hexane, and the product (93.5 mg., 90.7%) was eluted with 350-ml. of benzene. The product crystallized from hot glacial acetic acid by the dropwise addition of water to afford 68.6 mg. (66.6%) of IX as colorless plates, m.p. 243–246° with slight decomposition (Pyrex capillary). A sample for analysis was crystallized twice more from acetic acid–water and dried for 24 hours at 0.4 mm. (98°); m.p. 248–250.5° with slight decomposition (Pyrex capillary), $[\alpha]^{27}D$ +115.3° (*c* 0.72, α +0.83°).

Anal. Calcd. for $C_{36}H_{50}O_3$: C, 81.46; H, 9.50. Found: C, 81.56; H, 9.65.

The infrared absorption of the product in 5% carbon tetrachloride was identical with the starting material, except for a weaker absorption at 1425 $cm.^{-1}$. The optical rotatory dispersion curves of VIII b and IX are shown in Fig. 1.

Ursa-12:20(30)-dien-3β-yl Benzoate (X).—An ethereal solution of triphenylphosphine-methylene[31,32] was prepared by stirring a suspension of 1.9785 g. of triphenylmethylphosphonium bromide in 22 ml. of absolute ether with 3.4 ml. of ethereal *n*-butyllithium solution (1.51 *N*) under nitrogen at room temperature for 3 hours. To a solution of 52 mg. of 20-norurs-12-en-20-on 3β-yl benzoate in *ca.* 20 ml. of absolute ether under nitrogen was injected 2.5 ml. of the freshly prepared ethereal triphenylphosphine-methylene reagent with stirring. The mixture was stirred under nitrogen at room temperature overnight. The ether was stripped off and replaced with *ca.* 20 ml. of tetrahydrofuran (distilled from sodium and stored over sodium wire and calcium hydride), and the mixture was heated to reflux for 5.5 hours under nitrogen. The solvent was removed *in vacuo* on a steam-bath, and the residue was extracted into ether, washed with dilute hydrochloric acid, and worked up in the usual way. The crude product was placed on a column of alumina (10 g.). Hexane (100 ml.) eluted 15 mg. of an oil, and 30 ml. of benzene eluted 20.4 mg. of product X; infrared absorption of the product in 5% carbon disulfide: 1718(s) and 1277(s) $cm.^{-1}$ for benzoate; 1644(w) and 888(m) $cm.^{-1}$ for terminal methylene. A single recrystallization of 18 mg. of the product from ethanol–water afforded 11.5 mg. of colorless plates, m.p. 224–226° (hot-stage microscope).

3-Methylenecholestane.—To a solution of 200 mg. of cholestan-3-one in 15 ml. of absolute ether stirring under a nitrogen atmosphere was added 7.5 ml. of ethereal triphenylphosphine-methylene (2.0×10^{-4} mole/ml.). The mixture was stirred under nitrogen for 15 hours at room temperature. The ether was stripped off and replaced with tetrahydrofuran, and the mixture was heated to reflux for 8 hours under nitrogen. The product was worked up as described above (*cf.* preparation of 20-norus-12-en-20-on-3β-yl benzoate), was filtered in *n*-hexane solution over 15 g. of alumina and crystallized from ethyl acetate–methanol to give *ca.* 85 mg. of colorless needles, m.p. 64.5–65° (hot-stage); infrared absorption: 887(s), 1650(m), 3080(w) $cm.^{-1}$.

3β-Methylcholestane.—To a solution of 27 mg. of 3-methylenecholestane in 1.0 ml. of absolute dioxane was added 149 mg. of lithium and 3.0 ml. of ethylenediamine, the mixture was cooled in a water-bath until it was no longer exothermic (*ca.* 15 min.) and allowed to stand at room temperature for 15 hours. The mixture was then heated for 2 hours at 55–57°, diluted with water, and product was extracted into ether. The ether extract was washed with dilute hydrochloric acid and dilute sodium hydroxide solutions and then worked up in the usual way. Crystallization of the crude product from chloroform–methanol gave 20 mg. of colorless needles, m.p. 101–102° (hot-stage), $[\alpha]^{28}D$ +26° (*c* 0.96, α +0.25°). These constants are in agreement with those described by Barton[32] (m.p. 105–106°, $[\alpha]D$ +28°).

α-Amyrin Acetate.—To a solution of 101 mg. of 20-norurs-12-en-20-on-3β-yl benzoate (m.p. 246–49°, Pyrex capillary) in 25 ml. of absolute ether stirring under a nitrogen atmosphere was injected 9.0 ml. of ethereal triphenylphosphine-methylene (2.1×10^{-4} mole/ml.). The mixture was stirred for 15 hours at room temperature, the ether was stripped off, and the residue was heated to reflux for 7.75 hours with tetrahydrofuran under a nitrogen atmosphere. The mixture was concentrated to dryness, extracted into ether, washed with dilute hydrochloric acid and dilute sodium hydroxide solutions, and then worked up in the usual way. The crude product was heated to reflux for 11 hours under nitrogen with a mixture of 15 ml. of dioxane, 35 ml. of methanol and 3.6 g. of sodium hydroxide pellets. The solution was concentrated to dryness on a steam-bath in a stream of nitrogen, and the residue was extracted into ether and worked up in the usual way. The product, ursa-12:20(30)-dien-3β-ol, was separated from the triphenylphosphine oxide and other by-products by chromatographing the saponification mixture over 5 g. of alumina. The product (72 mg.)

(37) See T. R. Ames, J. L. Beton, A. Bowers, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.*, 1905 (1954).

(38) See D. H. R. Barton and K. H. Overton, *ibid.*, 2639 (1955).

(39) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, J. Wiley and Sons, Inc., New York, N. Y., 1947, pp. 607–609.

was eluted with 150 ml. of benzene and identified by its infrared absorption in carbon disulfide solution, which was void of any carbonyl absorption and which showed strong terminal methylene absorption at 887 cm.$^{-1}$.

A mixture composed of 63 mg. of crude ursa-12:20(30)-dien-3β-ol, 1.5 ml. of absolute dioxane, 5.0 ml. of ethylenediamine and 134 mg. of lithium was stored 13 hours at room temperature and was then heated to 58° for 3 hours. The mixture was diluted with water, and the product was extracted into ether and worked up in the usual way. The semi-solid product was treated with a mixture of 1.0 ml. of pyridine and 1.0 ml. of acetic anhydride for 45 min. on a steam-bath and then allowed to stand for 48 hours at room temperature. Methanol was added to the reaction mixture to decompose the excess acetic anhydride, and the mixture was concentrated to dryness. The residue was dissolved with a few drops of benzene and placed on a 2-g. column of alumina. Elution with 30 ml. of benzene afforded 54.5 mg. of crude α-amyrin acetate. One recrystallization from methylene chloride–methanol gave 30 mg. of colorless crystals, m.p. 209.5–212.5° (hot-stage). The melting point was raised to 217–221° with two more recrystallizations from the same solvents. A sublimation followed by a recrystallization from *n*-heptane raised the melting point to 223.5–225° (hot-stage). A mixed melting point with an authentic specimen of α-amyrin acetate (m.p. 225–226°) was 223.5–225° (hot-stage). The infrared absorption spectra of the authentic specimen and the synthetic product in carbon disulfide were identical. X-Ray powder diffraction patterns were prepared for both the synthetic product and the authentic specimen. The *d*-values are given in ångström units with the chromium radiation weighted wave length of 2.28962 Å. used as the basis of spacing calculations. Rotatory dispersion curves were run on both the synthetic and authentic specimens of α-amyrin acetate. The curves were identical.

Authentic specimen	Synthetic product	Authentic specimen	Synthetic product
15.41	15.41	4.35	4.34
9.18	9.29	3.80	3.82
7.74	7.74	3.47	3.47
6.45	6.50	3.31	3.32
5.76	5.76	3.07	3.07
5.40	5.42	2.98	2.99
4.65	4.60		

A second preparation of α-amyrin acetate from 55 mg. of 20-norurs-12-en-20-on-3β-yl benzoate (m.p. 250–252° on a hot-stage) afforded 43 mg. of crude ursa-12:20(30)-dien-3β-ol and 45 mg. of crude α-amyrin acetate. The product after one recrystallization from methylene chloride–methanol melted from 202–212° (hot-stage). A sublimation followed by two recrystallizations from *n*-heptane gave α-amyrin acetate, m.p. 223–225°.

URBANA, ILL.

[CONTRIBUTION FROM THE VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Reaction of Anhydrous Formic Acid with Proteins[1]

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RECEIVED SEPTEMBER 4, 1958

The effect of anhydrous formic acid on proteins and on serine containing peptides at 25° has been studied. In contrast to the action of concentrated mineral acids and in contrast to the interpretation of Josefsson and Edman, formic acid transforms the aliphatic hydroxyl groups into formate ester groups but does not cause any significant N → O-peptidyl shift.

Introduction

Elliott[3,4] clearly demonstrated that under the influence of concentrated sulfuric acid the peptide bonds involving the nitrogen of hydroxyamino acid residues in silk fibroin and lysozyme were transformed to O-ester linkages (N→O-peptidyl shift), and the amino groups of serine and threonine became free. Later McConnell and co-workers[5,6] applied this method to gluten and gliadin. Lucas and co-workers[7] showed that the peptidyl shift of serine residues in silk fibroin took place to almost the same extent in anhydrous phosphoric acid as in concentrated sulfuric acid. Recently Josefsson and Edman[8,9] reported reversible inactivation of lysozyme and ribonuclease due to N→O-peptidyl shift which resulted from incubating the proteins with anhydrous formic acid.

Since of the three acid reagents anhydrous formic acid seemed to be the mildest, the action of this acid on several proteins was tested. Contrary to expectation almost no increase of α-amino groups could be observed by the ninhydrin reaction after formic acid treatment of proteins. Furthermore, it was found that the action of formic acid on N-acetylseryltyrosine which was isolated from tobacco mosaic virus protein[10,11] did not produce an amino group in this peptide as would be expected if the acetyl shift had taken place and O-acetylseryltyrosine had been formed.

Consequently, in order to elucidate the reaction between formic acid and hydroxyamino acid residues in protein, the action of formic acid on N-acetylserine and on several proteins has been studied. The results which are described in the present paper show that formic acid does not act primarily as a catalyst for the N→O-acyl shift but as a formylating agent of the hydroxyl group of serine (and threonine) residues in peptide linkage.

Experimental

Formic Acid.—98–100% formic acid (Merck) was dried over boric anhydride for a week and distilled over anhydrous copper sulfate *in vacuo*. Formic acid-C^{14} was pre-

(1) Aided by United States Public Health Service Training Grant CRTY-5028.

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