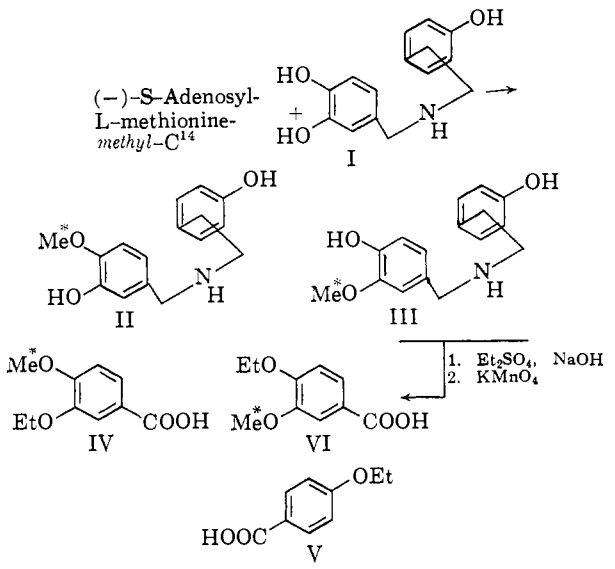

methionine sulfone, and choline are without effect. Monomethylated derivatives II and III are not further methylated.

Norbelladine (I, 250 mμmoles) was incubated at pH 8.1 for 5 hr. at 26° with (−)-S-adenosylmethionine-*methyl*-C^{14} (150 mμmoles, specific activity, 9.2 c./mole) in the presence of *Nerine* enzyme (4.3 mg. of protein). Radioactive material was then extracted into ethyl acetate by adjusting the reaction mixture to pH 10. Separate portions of this extract were used to determine the extent of *para* and *meta*-O-methylation of the catechol moiety of norbelladine (I).

Synthetic N-isovanillyltyramine (II)[2] (m.p. 183–185°; *Anal.* Found: C, 70.09; H, 7.03) was added to one aliquot; 78% of the radioactivity could not be separated from this compound upon repeated crystallization from ethanol. To rule out possible contamination with isomer III, the recrystallized material was degraded *via* O-ethylation with diethyl sulfate and alkali followed by oxidation with potassium permanganate. The resulting acids were converted to their methyl esters with diazomethane and subjected to preparative gas chromatography. This procedure separated the non-radioactive ester of acid V (acid m.p. 195–196°[3]) from a fraction containing a possible mixture of the esters of acids IV and VI. The latter fraction was hydrolyzed and acid IV, m.p. 165–166°,[4] was freed from any trace of acid VI, m.p. 193–194°[5] (see below) by repeated recrystallization to the same constant molar specific radioactivity as phenol II.

To determine the amount of *meta*-methylation, N-vanillyltyramine hydrobromide (III-hydrobromide, m.p. 158–162°; *Anal.* Found: C, 54.09; H, 5.69) was added to another aliquot of the radioactive extract. This material could not be recrystallized to constant specific activity, since isomers II and III (as hydrobromide) co-crystallized. Accordingly, this mixture was degraded as described above. Acid VI was freed from traces of radioactive acid IV by repeated recrystallization and finally retained only 3.5% of the original radioactivity of the extract.

These results indicate that the ratio of *para* to *meta*-methylation of norbelladine catalyzed by the *Nerine* enzyme is 22:1. Approximately 4% of the initial norbelladine had been methylated, although no attempt was made to obtain complete consumption of either substrate.

Another enzyme, present in rat liver, also catalyzes the methylation of catechols using (−)-S-adenosyl-L-methionine as a methyl donor.[6] The products of this enzyme have been shown to be predominantly *meta*-O-methylated isomers.[7] Rat liver enzyme (4.5 mg. of protein) was incubated for 4 hr. at 37° under conditions similar to those used for the plant enzyme, but in the presence of 5×10^{-4} *M* magnesium chloride. The *para* to *meta* ratio (II:III) was found to be 0.28:1, indicating that the highly specific *para*-methylation is a property of the plant enzyme. Approximately 18% of the norbelladine had been methylated. Barton, Kirby and Taylor have shown recently[8] that the *para*-O-methylated phenol II was converted into haemanthamine without loss of the methoxyl carbon, *i.e.*, cyclization of the methoxyl to a methylenedioxy group had occurred. The *meta*-O-methylated isomer III apparently was not tested. The results reported herein suggest that the main biosynthetic route from norbelladine to haemanthamine proceeds by way of II rather than III. The findings suggest also that (−)-S-adenosyl-L-methionine is the source of the methylenedioxy groups in the Amaryllidaceae alkaloids, and are in accord with the earlier observation by Scribney and Kirkwood,[9] who found that the methyl carbon of L-methionine was a precursor of the methylenedioxy group of the Papaveraceae alkaloid protopine.

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RECEIVED MAY 17, 1963

The Synthesis of 3-β-D-Ribofuranosyladenine[1]

Sir:

Reports of the isolation of naturally occurring 3-substituted purines, triacanthine (6-amino-3-(γ,γ-dimethylallyl)-purine)[2-4] and 3-ribosyluric acid,[5] stimulated us to synthesize the isomer of adenosine, 3-ribofuranosyladenine (Ia), in order to compare its behavior in chemical and biological systems with that of adenosine, for example, to see whether adenosine and "3-isoadenosine" exhibit a relationship similar to that of uridine and pseudouridine.[6]

Direct alkylation on the 3-position of adenine has recently been shown to be not only a possibility but a preference,[2,7-9] and we are now able to describe a

(1) Supported in part by a Research Grant (USPHS-RG5829, currently GM-05829-05) from the National Institutes of Health, U. S. Public Health Service.

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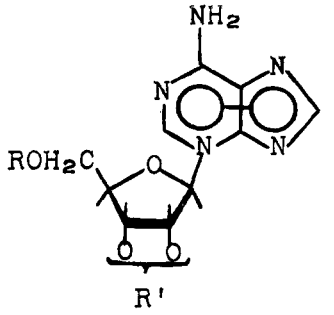
(9) 3-Allylation and 3-benzylation by T. Fujii, University of Illinois, reported by N. J. Leonard at The International Symposium on Organic

parallel synthesis of 3-ribofuranosyladenine. When adenine was allowed to react with 1-bromo-2,3,5-tribenzoyl-D-ribofuranose in acetonitrile at 50° for 36 hr., 3-(2′,3′,5′-tribenzoyl-D-ribofuranosyl)-adenine (Ib) was obtained in 25% yield. In a similar manner 3-2′,3′,5′-triacetyl-D-ribofuranosyl)-adenine (Ic) was prepared. Removal of the protecting groups from Ib or Ic in methanolic ammonia yielded 3-ribofuranosyladenine (Ia). A second alkylated product also resulted, as exemplified by the co-formation of 2′,3′,5′-tribenzoyladenosine (18%), along with 25% of Ib and 35% recovery of adenine. Conversion of the tribenzoyl derivative to adenosine established structure and completed the synthesis of this nucleoside directly from adenine. This facile synthesis is applicable to isotopic labeling[10] and is competitive with the syntheses which employ blocking groups on the purine nucleus and the silver or chloromercury salt.[11]



	R	R′
Ia	H–	2H–
b	C_6H_5CO–	$2C_6H_5CO$–
c	CH_3CO–	$2CH_3CO$–
d	H–	$CH_3C(CH_3)$

For the major product, the position of substitution on adenine of the sugar moiety was established as N-3 by comparison of the ultraviolet spectra and pK_a' values of I (Table I) with substituted adenines of known structure.[2,3] Although it seemed probable that alkylation with the protected ribofuranosyl halide would lead to the β-configuration at C-1′ in Ia, because of the participation of the acyloxy group at C-2[12,13] and because the adenosine derivative (which has the β-configuration)[14] is formed in the same reaction, direct evidence was sought from n.m.r. spectroscopy. In adenosine and 3-ribofuranosyladenine and their derivatives, H_1' appears as a doublet, well separated from other peaks in the spectrum. The coupling constants of the nucleosides and their derivatives were determined, and the dihedral angles (ϕ) between the intersecting planes defined by H_1'–C–C and C–C–H_2' were calculated by the Karplus approximate equations[15] for angles between 0 and 90° and 90 and 180° (Table II).

TABLE I

PHYSICAL PROPERTIES OF 3-RIBOFURANOSYLADENINE AND DERIVATIVES[a]

Compd.	M.p., °C.	Ultraviolet spectra[b] λ_{max}, mμ	ϵ_{max}	λ_{min}, mμ	Solvent	pK_a'[c] H_2O	pK_a'[c] 50% DMF
Ia[d]	210–211	277	10,900	243	H_2O, pH 7	5.5	4.7
	dec.	274	13,900	236	0.1 *N* HCl		
Ib	246–247	282	13,300	252	EtOH	...	4.9
		277	20,900	251	EtOH, 0.1 *N* HCl		
Ic	224–225	275	11,500	242	H_2O, pH 7	5.6	4.8
		275	13,200	237	0.1 *N* HCl		
Id	250–251	277	12,900	245	H_2O, pH 7		
		274	18,200	238	0.1 *N* HCl		

[a] Satisfactory analytical results (C, H, N) were obtained for each compound. [b] Obtained on a Cary Model 14 recording spectrophotometer. [c] We are indebted to Dr. Harold Boaz, Eli Lilly and Co., Indianapolis, Ind., for the pK_a' determinations. [d] Hydrochloride: $[\alpha]^{28}D$ −30° (*c* 2.95, H_2O); plain negative rotatory dispersion curve.

TABLE II

COUPLING CONSTANTS AND CALCULATED DIHEDRAL ANGLES[a] FOR DERIVATIVES OF ADENOSINE AND 3-RIBOFURANOSYLADENINE

Derivative	Adenosine $J_{H_1'H_2'}$[b]	Adenosine ϕ_{trans}	Adenosine ϕ_{cis}	3-Ribofuranosyladenine $J_{H_1'H_2'}$[b]	3-Ribofuranosyladenine ϕ_{trans}	3-Ribofuranosyladenine ϕ_{cis}
Unsubstituted	5.2	139°		4.5	135°	41°
α-Adenosine[c]	5.1		37°			
2′,3′,5′-Triacetyl-	4.7	136°		3.8	131°	44°
2′,3′-Isopropylidene-	2.9	125°		2.2	121°	57°

[a] See ref. 15. [b] N.m.r. spectra of the hydrochloride salts in D_2O were determined on the Varian A-60 spectrometer; *cf.* ref. 2 and L. Gatlin and J. C. Davis, Jr., *J. Am. Chem. Soc.*, **84**, 4464 (1962). *J* values are in c.p.s. A complete discussion of spectra will be included in subsequent publications. [c] Generously provided by Professor H. G. Khorana, Institute for Enzyme Research, University of Wisconsin, Madison, Wis.

No safe conclusions could be drawn from $J_{H_1'H_2'}$ of the unsubstituted nucleosides,[16] since the observed coupling constants predict angles which can be accommodated by structures in which H_1' and H_2' are either *cis* or *trans*.[17,18] For example, adenosine and α-adenosine (9-α-D-ribofuranosyladenine)[19] have nearly identical coupling constants (Table II). However, Jardetzky observed that when the ribofuranose ring is constrained by fusion with a second ring, as in certain nucleoside cyclic phosphates, $J_{H_1'H_2'}$ is reduced.[20] If the value is low enough, assignment of configuration is possible.[17,20a] The coupling constant for 3-(2′,3′-isopropylidene-D-ribofuranosyl)-adenine (Id)[21] was observed to be 2.2 c.p.s., which corresponds to a dihedral angle of about 121° if H_1' and H_2' are *trans*, and 57° if *cis*. Models of Id indicate angles of about 120 and 0° for the β- and α- configurations, respectively. Considerable distortion would have to be introduced into the rings to produce an angle of 57° (calculated for α-configuration), and we therefore conclude that Ia is a β-nucleoside. We were also guided to this conclusion by the fact that $J_{H_1'H_2'}$ for 2′,3′-isopropylideneadenosine, the configuration of which is known to be β,[14] is 2.9 c.p.s., corresponding to an angle of 125°.

In preliminary experiments to assess the biological activity of the adenosine isomer in suitable bacterial and mammalian cell systems,[22] it was observed that "3-isoadenosine" is capable, as is adenosine, of supporting the growth of an adenine-requiring *E. coli* mutant.[23]

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(22) Personal communication from P. J. Simpson and G. B. Boder, Division of Biology and Pharmacology, Lilly Research Laboratories, Indianapolis 6, Indiana, to whom we are happy to extend our thanks. Details of the biological observations will be published shortly.

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In contrast, it was found that "3-isoadenosine" is unable to replace adenosine in supporting the growth of mammalian cells cultures[24] in a system requiring an exogenous purine source[25] for optimal growth.

Forthcoming reports from our Laboratory will discuss the work in detail, the phosphorylation of 3-β-D-ribofuranosyladenine or "3-isoadenosine," and its conversion to other derivatives isomeric with those of adenosine.

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RECEIVED MAY 2, 1963

Microwave and Mass Spectra of Sulfur Monofluoride[1]

Sir:

The preparation of S_2F_2 has been claimed by a number of investigators.[2-5] More recently an infrared spectrum was assigned to this species.[6] However, doubts have been expressed about the characterization[7,8] and existence of S_2F_2.[9] Using methods similar to those of the earlier workers, we have prepared S_2F_2 and have positively identified it as S_2F_2 by means of its mass and microwave spectra. We further determined the structure which turns out to be an analog of the well known SOF_2.

Approximately 1 g. of argentous fluoride (AgF) and 6 g. of sulfur were mixed and slowly heated *in vacuo* in glass to the melting point of sulfur. The gases produced were condensed with liquid N_2. Microwave absorption lines were observed due to SO_2 and SOF_2 as well as additional lines. The mass spectrum indicated SiF_4, SO_2 and SOF_2 as well as peaks at 83 and 102 m/e units. The analysis of the mass spectrum, taking into account the presence of SiF_4, SO_2 and SOF_2 and the expected isotopic contributions from sulfur, led to the tentative conclusion that the new microwave lines were due to S_2F_2.

Drying of AgF *in vacuo* reduced the SO_2, SiF_4 and SOF_2 content and increased the S_2F_2 yield. It also brought in microwave and mass peaks due to SF_4. Low temperature fractional distillation produced a sample which we estimate contained approximately 90% S_2F_2 with SO_2, SOF_2 and SF_4 as impurities. The relative mass spectral cracking pattern obtained after correction for SO_2, SOF_2 and SF_4 is reported in Table I.

The microwave spectrum of S_2F_2 was observed and assigned to transitions, largely with the aid of the Stark patterns and the fit to a rigid rotor formula (see Table II). In addition to the spectrum of the main species, weaker satellite lines were found which had approximately the correct relative intensity to be sulfur-34 lines and the correct temperature dependence of intensity (also see Table II). Table III shows the rotational constants and moments of inertia extracted from the analyzed spectra. These were used to determine the structure of S_2F_2, applying several alternative standard methods. The bond distances and angles shown in Fig. 1 carry limits of error which generously cover the variations among these methods.

TABLE I

RELATIVE MASS SPECTRAL CRACKING PATTERN FOR S_2F_2[a]

Mass/charge	Relative abundance	Assignment
102	100	$S_2F_2^+$
83	30.2	S_2F^+
70	7.4	SF_2^+
64	23.2	S_2^+
51	6.6	$SF^+(S_2F_2^{++})$
32	26.6	$S^+(S_2^{++})$
19	2.6	F^+
41.5	~0.6	S_2F^{++}
35	<0.1	SF_2^{++}

[a] Obtained with Consolidated Engineering Corporation spectrometer Model 21-103c at 70 ionizing volts and 10-μa. ionizing current.

TABLE II

MICROWAVE SPECTRUM OF S_2F_2[a]

Transition	Observed	Calculated
	$S^{32}S^{32}F_2$	
$0_{00} \rightarrow 1_{10}$	12147.40	12147.27
$1_{01} \rightarrow 2_{11}$	20083.62	20083.57
$1_{10} \rightarrow 2_{11}$	14937.61	(14937.61)[b]
$1_{11} \rightarrow 2_{12}$	13067.63	(13067.63)
$2_{12} \rightarrow 2_{20}$	15577.32	15576.99
$1_{11} \rightarrow 2_{21}$	28505.50	(28505.50)
$2_{11} \rightarrow 3_{21}$	34312.85	34313.12
$3_{22} \rightarrow 3_{30}$	23486.60	23486.75
$4_{23} \rightarrow 4_{31}$	23915.70	23916.24
	$S^{32}S^{34}F_2$	
$0_{00} \rightarrow 1_{10}$	12068.54	(12068.54)
$1_{01} \rightarrow 2_{11}$	19969.03	(19969.03)
$1_{11} \rightarrow 2_{21}$	28304.60	28305.14
$2_{12} \rightarrow 3_{22}$	36205.45	36205.62
$2_{11} \rightarrow 3_{21}$	34109.39	34108.86
$3_{22} \rightarrow 3_{30}$	23230.90	(23230.90)
	$S^{34}S^{32}F_2$	
$0_{00} \rightarrow 1_{10}$	12007.65	(12007.65)
$1_{01} \rightarrow 2_{11}$	19676.66	(19676.66)
$1_{11} \rightarrow 2_{21}$	28353.30	28353.95
$3_{22} \rightarrow 3_{30}$	23972.04	(23972.03)
$4_{23} \rightarrow 4_{31}$	24341.36	24340.95

[a] Obtained with a conventional Stark modulated spectrometer. Frequencies reproducible to ±0.1 Mc. [b] Transitions in parenthesis used to calculate rotational constants.

This structure is very similar to that of SOF_2 in which[10] the sulfur–fluorine distance is 1.58 Å., angle FSF is 92.8° and angle FSO is 106.8°. The sulfur–sulfur distance in S_2 is reported[11] to be 1.89 Å.

The basis for asserting that the above microwave spectrum is due to S_2F_2 is as follows. (1) The intensity of these microwave lines obtained from different samples with different purification steps correlate with mass spectral analysis indicating the amount of S_2F_2. (2) The structure obtained by assuming that the empirical composition is S_2F_2 is almost identical with that predicted from the known structure of SOF_2.

(1) This work was made possible by support extended Harvard University by the Office of Naval Research.

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